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EFFECTS OF EXERCISE ON THE CORONARY BLOOD FLOW, HEART RATE AND BLOOD PRESSURE OF TRAINED DOGS WITH DENERVATED AND PARTIALLY DENERVATED HEARTS

HIRAM E. ESSEX, J. F. HERRICK, EDWARD J. BALDES AND FRANK C. MANN

From the Division of Experimental Medicine, Mayo Foundation, and Division of Physics and Biophysical Research, Mayo Clinic and Mayo Foundation, Rochester Minnesota

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In a previous paper (1) we have reported simultaneous observations on the blood pressure, heart rate and coronary blood flow of the dog in response to exercise on a treadmill. It was shown that the changes of heart rate at the beginning of exercise were usually a better criterion of the changes of coronary blood flow than the arterial blood pressure. With the beginning of exercise a rapid increase of coronary blood flow was seen which was accompanied by a greatly accelerated pulse. It has been shown in a relatively large series of experiments that the behavior of the blood pressure is unpredictable during the initial minutes of exercise. It may increase, decrease, be diphasic or remain unchanged. This behavior of the blood pressure supported the conclusion that the increased coronary blood flow observed at the beginning of exercise was not the result of changes of the arterial blood pressure alone.

In another study (2) it was demonstrated that increasing or decreasing the cardiac rate while keeping other factors relatively constant resulted respectively in an increased or decreased coronary blood flow.

The sudden acceleration of the heart in dogs coincident with the beginning of exercise is considered to be owing principally to a reduction of cardiovagal tone (3). It was therefore considered important to study the effects of exercise on the heart rate, blood pressure and coronary blood flow of dogs after denervation and partial denervation of the heart.

METHODS. Denervation of the heart was done in stages. Right vagotomy was usually done first. Through an incision low in the neck and over the anterior aspect of the thorax the vagus nerve was exposed. This nerve and the brachial artery were mobilized in such a manner as to make possible the identification and section of all the branches of the vagus except the recurrent nerve, which was preserved. At the second stage sympathetic ganglionectomy was done on the left side from the eighth costal interspace anteriorly including the

stellate ganglion. The third stage consisted of the same operation on the sympathetic ganglia of the right side. In the fourth stage the left vagus nerve was sectioned about midway in the neck. When it was desired only to vagotomize the heart the two operations were done as just described in stages one and four.

The coronary blood flow was measured by the thermostromuhr method. In all experiments the unit was applied to the circumflex branch of the left coronary artery before or after the last stage of cardiac denervation; that is, before or after sectioning the remaining vagus nerve. By applying the unit before sectioning the remaining vagus nerve, control data on blood flow, blood pressure and heart rate obtained on a partially denervated heart could be compared with those from a totally denervated heart. When data on animals on which sympathetic ganglionectomy alone had been performed were desired both operations were done before the thermostromuhr unit was applied to the coronary vessel.

In the experiments on animals that had vagotomized hearts both nerves were sectioned in some cases while in other cases only the right vagus was sectioned before the application of the thermostromuhr unit. Thus in the latter experiments the effects of the loss of one vagus could be compared with the results following total vagotomy.

All of the major operative procedures were done with the animals under general anesthesia and sterile technic was observed. The blood pressure was recorded optically from a cannulated femoral or carotid artery using a glass spoon manometer (4). The heart rate was recorded by an electrocardiograph. The dogs were trained to exercise on the treadmill before and during their preparation for the various experiments.

Except in cases of persistent vomiting such as occurred in the vagotomized animals, when repeated feeding was necessary, the dogs were fed each day after the observations had been completed. Food was withheld at other times.

Routinely the animals were exercised for a total of fifteen minutes: five minutes with the treadmill running horizontally, five minutes with it elevated at about an angle of 15 degrees and five minutes again in the horizontal position.

RESULTS. *Cardiac sympathectomy.* A series of experiments was done on animals 24 to 124 days following the first operation in which sympathetic ganglionectomy was done. The question of whether regeneration of the sympathetic nerves had occurred could not justifiably be raised concerning experiments that had been done within twenty-four days after the first stage of cardiac sympathectomy. The results of all such experiments were not essentially different from those obtained previously on dogs that had intact nervous systems. Coronary blood flow was markedly increased with increments of the rate of work. As in experiments reported previously (1) increases of the coronary blood flow sometimes occurred in the presence of a decreased blood pressure. Increases of coronary blood flow were always associated with an increased heart rate. This has been taken as indicating that factors associated with heart rate alone are capable of profoundly influencing coronary blood flow in a manner that is thus far unexplained (table 1).

Bilateral cervical vagotomy. In some experiments both vagus nerves were

TABLE 1

Effect of exercise on heart rate, coronary blood flow and blood pressure of dogs after cardiac sympathectomy

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
									6–5–40: Left cardiac sympathectomy 8–7–40: Right cardiac sympathectomy 10–9–40: Unit applied
1	Heart rate	90	120	160	140	130		130	10–14–40: Flow was 115 cc. per minute, 10 seconds after treadmill was elevated
	Blood flow*	70	115	132	115	89	85	82	
	Blood flow	69	90	145	120	105	82		10–15–40: Left femoral artery cannulated
	Blood pressure	101	97	111	132	121			
									12–18–40: Left cardiac sympathectomy 1–8–41: Right cardiac sympathectomy 2–26–41: Unit applied
2	Blood flow	70	137	162	155	120	108		3–6–41: Left femoral artery cannulated
	Blood pressure	108	102	119	127	111	110		
									1–18–40: Left cardiac sympathectomy 1–8–41: Right cardiac sympathectomy 2–6–41: Unit applied
3	Heart rate	130	185	190	190	160	150		2–8–41
	Blood flow	118	285	215	205	170	118		
	Heart rate	110	160	190	170	130	120		2–10–41
	Blood flow	65	170	215	200	105	76		
	Heart rate	130	180	210	220	180	175	140	2–12–41: Left femoral artery cannulated
	Blood flow	98	155	190	190	160	170	89	
	Blood pressure	130	160	162	162	?	170	151	

* Blood flow = cc. per minute.

sectioned as long as thirty days before the application of the thermostromuhr unit. These animals had fully recovered from the effects of the operation itself but continued to vomit several times a day as long as they lived. In spite of the persistent vomiting the animals, when fed frequently, retained enough food to maintain the body weight near the control value.

In agreement with the findings of Samaan (3) the heart rate increased to about 180 beats each minute immediately after sectioning the second vagus nerve which was usually done under infiltration anesthesia with procaine hydrochloride. When the remaining vagus nerve was sectioned an increase of coronary blood flow always occurred. In one experiment a marked transient elevation of blood pressure was observed following section of the second vagus nerve. As a rule the increased heart rate remained maximal for only a few minutes after the second vagus was cut. The heart rate gradually decreased and twenty to thirty minutes after total vagotomy it had usually stabilized at about 140 to 150 beats each minute. The resting heart rate did not in every case remain indefinitely at about 140 beats each minute but continued in some animals to decrease gradually over a period of several weeks until it had reached 120 to 90 beats each minute. That this decreased rate was not owing to a restoration of vagal influence was demonstrated by the inability of intravenously administered atropine sulfate, in doses of 0.1 to 0.2 mgm. for each kilogram of body weight to affect the heart rate. In some animals the heart rate and coronary blood flow slowly decreased over a period of several days following section of the second vagus nerve, but the values obtained before sectioning the second vagus were not usually reached.

Simultaneously with the beginning of exercise the heart with one or both vagi invariably accelerated greatly, frequently doubling its control rate within thirty seconds. The coronary blood flow at the same time increased rapidly, sometimes showing increases of nearly 100 per cent. The initial responses of the blood pressure were unpredictable. After total vagotomy the heart lost its power of marked acceleration at the beginning of exercise or with increasing rate of work. Ten to twenty beats each minute was usually the limit of acceleration in either case. In general in the absence of an increased heart rate but in the presence of a much elevated blood pressure the coronary flow showed large increases. On the other hand, in the presence of a slightly decreased or a constant blood pressure and in the absence of cardiac acceleration the coronary flow remained practically unchanged. After vagotomy the coronary blood flow at the beginning of exercise was apparently influenced more by the arterial blood pressure than by any other single factor (table 2).

Total cardiac denervation. Observations were made on animals whose hearts had been totally denervated, the operative procedures for which have already been described. By applying the thermostromuhr unit to the coronary artery after cardiac sympathectomy and unilateral vagotomy or previous to sectioning the remaining vagus nerve, control data on the totally denervated hearts were obtained.

It has been shown in another section of this paper that the response of the blood pressure, heart rate and coronary blood flow to exercise in animals that had

TABLE 2

Effect of exercise on heart rate, coronary blood flow and blood pressure of vagotomized dogs

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
									3–27–40: Sectioned left vagus 4–17–40: Sectioned right vagus 5–15–40: Unit applied
4	Heart rate	110	130	150	130	120		120	5–20–40
	Blood flow*	48	90	145	82	63		64	
	Heart rate	140	140	150	140	140		140	5–21–40: Number 1 left femoral artery cannulated
	Blood flow	62	122	136	122	80		65	
	Blood pressure	100	140	145	135	112		100	
	Heart rate	110	130	140		130	130		5–21–40: Number 2
	Blood flow	62	96	136	122	62			
	Blood pressure	85	100	118	106	90			
									6–18–40: Sectioned right vagus 11–20–40: Unit applied
5	Heart rate	70	130	180	150	110	125		11–26–40: Control
	Blood flow	98	218	390	180	113	113		
	Heart rate	180	190	220	190	180	180		11–27–40: After sectioning left cervical vagus
	Blood flow	163	280	?	215	180	155		
	Heart rate	170	170	180	170	160	170		11–28–40
	Blood flow	120	165	235	125	108	105		
	Heart rate	150	160	180	160	150	150	150	11–29–40
	Blood flow	125	275	?	125	110	106		
	Heart rate	160	170	175	170	150	160		12–2–40: Left femoral artery cannulated
	Blood flow	145	192	395	210	96	102		
	Blood pressure	93	121	153	153	114	110		

* Blood flow = cc. per minute.

sympathectomized hearts was the same as seen in those that had intact sympathetic nervous sytems. The evidence secured in the present series of experi-

ments is confirmatory of this finding. The results of observations made on animals on which bilateral sympathectomy and unilateral vagotomy had been performed were indistinguishable from the results obtained on animals that had intact nervous sytems (upper tracing in fig. 1). When the remaining vagus was sectioned a profound difference in the results during exercise was seen (fig. 1). In brief, the results were strikingly like those described in experiments in which only bilateral cervical vagotomy had been done.

In one experiment the blood pressure was recorded before, during and after section of the remaining vagus nerve. In a control observation the blood pressure decreased slightly when exercise was begun while the coronary blood flow definitely increased but the heart rate accelerated only 20 beats each minute. The typical response of blood pressure, heart rate and coronary flow followed an augmentation of the rate of work. After recovery from the effects of this period of exercise the remaining vagus was isolated and prepared for sectioning. Following a control period of five minutes of exercise the remaining vagus was cut. The blood pressure rose immediately from 125 to 170 mm. of mercury. The heart rate increased 40 beats each minute and the coronary blood flow increased about 100 per cent. The increased heart rate remained maximal for only a few minutes, having decreased 10 beats each minute five minutes following section of the vagus. After an interval of five minutes the treadmill was again started in the horizontal position. The effect on the blood pressure was practically negative. The heart rate increased 10 beats each minute. The coronary blood flow was practically unaffected by the exercise. Five minutes later the rate of work was augmented by elevating the treadmill. The immediate effect on the blood pressure was a decrease which was reflected in the coronary blood flow. After a minute the blood pressure and coronary blood flow had increased above the control and the heart rate had accelerated to 190 beats each minute. A few days later the heart rate had decreased to 90 beats each minute when the animal was at rest (dog 8 in table 3).

Rectal temperature and exercise. Since changes of body temperature influence the rate of the heart it seemed important to determine the effect on temperature that resulted from the standard rate of exercise. The rectal temperature was taken before exercise and one minute after the treadmill was stopped at the completion of the usual exercise of fifteen minutes' duration. In nine experiments on sympathectomized dogs the increase of temperature was from 1 to 1.8°F. The average control temperature was 102.5°F., while the average at the completion of exercise was 103.6°F. In six observations on animals all of whose cardiac nerves except the left vagus had been removed the increase of temperature after exercising for fifteen minutes varied from 0.6 to 2.8°F. The average control temperature was 101.6°F. and the average temperature at completion of exercise was 103.1°F. Seven observations on vagotomized dogs showed increases from 0.4 to 2.8°F. resulting from periods of exercise of fifteen minutes' duration. Increases of temperature as great as just indicated undoubtedly cause an acceleration of the heart. It is doubtful whether the increased temperature accompanying the first 90 to 120 seconds of exercise was sufficient to

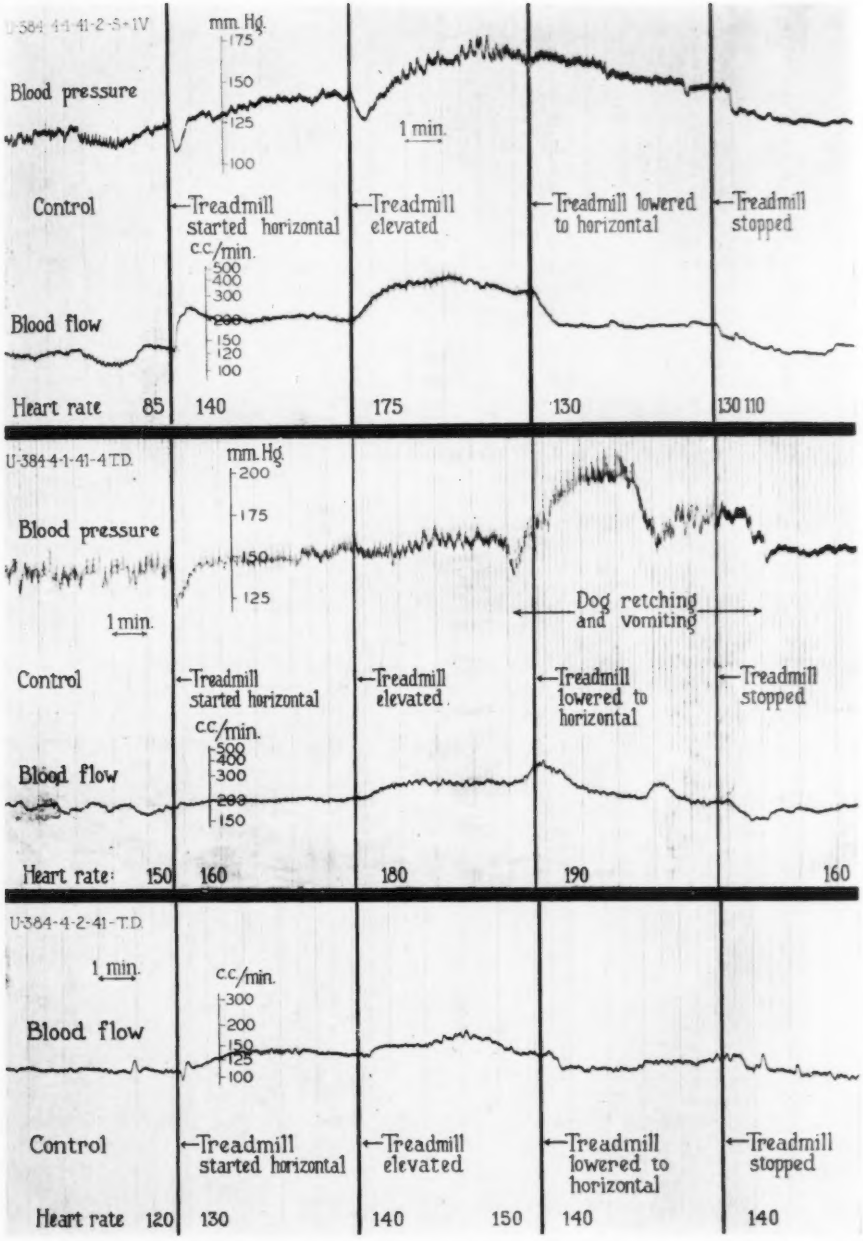

Fig. 1. The response of the blood pressure, coronary blood flow and heart rate of a dog after, first, partial and then total denervation of the heart. In the upper tracing the left vagus nerve is still intact. The middle tracing was taken thirty-five minutes after sectioning the left vagus nerve. The bottom record of coronary blood flow was made about twenty-four hours later.

TABLE 3

Effect of exercise on heart rate, coronary blood flow and blood pressure having partially an totally denervated hearts

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
									8–28–40: Right cervical vagotomy 9–11–40: Left cardiac sympathectomy 10–16–40: Right cardiac sympathectomy 11–6–40: Unit applied
6	Heart rate	90	130	170	140	110		100	11–13–40: Heart denervated except left vagus
	Blood flow*	29	53	90	94	80		66	
	Heart rate	130	150	200	200	170			11–15–40: Left vagus sectioned, total cardiac denervation
	Blood flow	42	57	105	67	55	51		
	Heart rate	130	150	185	160	140			11–16–40
	Blood flow	34	45	70	49	46	36		
									1–16–41: Right cervical vagotomy 1–29–41: Left cardiac sympathectomy 2–13–41: Right cardiac sympathectomy 3–19–41: Unit applied
7	Heart rate	105	140	180	155	130	110		3–25–41: Heart denervated except for left vagus
	Blood flow	150	190	?	206	175	171		
	Heart rate	100	130	170	160	140	120		3–26–41: Maximal flow with treadmill elevated 340 cc. per minute
	Blood flow	143	165	290	165	142	146		
	Heart rate	90	130	165	150	120	110		3–27–41
	Blood flow	165	178	285	185	132	150		
	Heart rate	75				100	100		3–31–41: Number 1
	Blood flow	103	180	220	144	115	118		

TABLE 3—(*Concluded*)

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
	Heart rate	75	130	170	140	115	100		3-31-41: Number 2
	Blood flow	109	185	275	175	128	143		
	Heart rate	70	130	160	120	110	90		4-1-41: Number 1
	Blood flow	112	165	235	150	113	112		
	Heart rate	85	140	175	130	110	100		4-1-41: Number 2 Left carotid cannulated
	Blood flow	137	215	340	190	150	137		
	Blood pressure	121	129	140	160	128	120		
	Heart rate	150	160	180	190	160	160		4-1-41: Twenty-five minutes after sectioning left vagus
	Blood flow	185	205	260	275	165	210		
	Blood pressure	141	147	151	193	159	151		
	Heart rate	120	130	140	140	140	130		4-2-41
	Blood flow	108	127	150	113	108	100		
									1-16-41: Right cervical vagotomy 1-29-41: Left cardiac sympathectomy 2-13-41: Right cardiac sympathectomy 3-19-41: Unit applied
8	Heart rate	130	140	190	170	140	130		3-25-41
	Blood flow	172	210	300	260	185	171		
	Heart rate	110	130	180	170	140	140		3-26-41
	Blood flow	150	165	250	225	174	165		
	Heart rate	130	150	180	170	140	130		3-27-41
	Blood flow	160	200	256	215	185	185		
	Heart rate	100	120	170	160	130	120	110	3-28-41
	Blood flow	165	225	330	295	280	247	230	
	Blood pressure	121	113	135	149	143	139	141	Left carotid artery cannulated 3-28-41: Left vagus sectioned
	Heart rate	90	100	140	120	110	100		3-31-41
	Blood flow	68	76	120	111	96	76		
	Heart rate	100	120	140	130	110	110		4-1-41
	Blood flow	104	104	130	122	120	110		
	Heart rate	95	100	130	120	110	100		4-2-41
	Blood flow	100	110	130	125	105	95		

* Blood flow = cc. per minute.

affect the heart rate or coronary blood flow materially but the elevated temperature was undoubtedly one of the factors responsible for the augmented pulse rate resulting from increments of the rate of work.

COMMENT. It must not be overlooked that the basal or resting heart rate and coronary blood flow of the recently vagotomized and totally denervated hearts were sometimes nearly twice as great as before denervation. It appeared that these hearts were being supplied with more blood than was required for their basal needs. When exercise was begun there was sufficient flow of blood to meet the needs of the heart in some cases for as long as five minutes. With further increments of the rate of work additional coronary blood flow resulted from increases of arterial blood pressure or from the operation of other mechanisms. The influence of increases of body temperature, the accumulation of metabolites and the rôle of hormones must be considered as possible factors in the production of the increased coronary blood flow that resulted from high rates of work continued for several minutes. It is doubtful whether these last-named factors played an important part in the increased coronary flow sometimes seen during the first ten to thirty seconds of exercise.

The nervous control of the coronary blood vessels has been under discussion for many years but complete agreement has not been reached on any particular phase of the problem. This is owing in part at least to the difficulty of devising and executing crucial experiments as well as repeating in all respects the work of others, and also in part to lack of complete confidence in any method yet developed for measuring coronary blood flow. In the present state of the science of physiology most methods at best probably give only a very rough approximation of what is taking place in the undisturbed organ.

With all the limitations acknowledged with respect to the present experiments, which have been in progress for a number of years, we submit that sufficient data are presented in this report to indicate rather conclusively the dominant rôle of the vagus in control of cardiac circulation. In not a single instance has there been any evidence that the sympathetic nerves exercised a tonic action on the heart or coronary vessels. A sympathectomized heart was indistinguishable from a normal heart as to rate and coronary blood flow. In every series of experiments the tonic action of the vagus was strongly evident. A dramatic change of rate or coronary flow was not seen except when the heart was deprived of both vagus nerves. So long as one vagus supplied the heart its reaction to exercise as respects rate and coronary flow was indistinguishable from that of the fully innervated heart. In the absence of the cardiac sympathetic nerves the sectioning of the remaining vagus nerve was followed by marked acceleration of heart rate and augmentation of coronary blood flow. The response of the rate and coronary blood flow of the totally denervated heart to moderate exercise was in many instances completely negative whereas the fully innervated heart showed marked acceleration and increased coronary blood flow with the same rate of exercise. The results of our experiments on the trained dog strongly support the findings of Anrep and Segall (5) on the innervated and denervated heart-lung preparation as regards the tonic vagal control of coronary blood flow and like-

wise our results support the findings of Samaan (3) with regard to the influence of the vagus on the heart rate of exercising dogs.

SUMMARY AND CONCLUSIONS

Observations have been made on the coronary blood flow, heart rate and blood pressure of trained dogs after the following procedures: 1, bilateral sympathetic ganglionectomy, from the eighth costal interspace anteriorly including the stellate ganglion; 2, double cervical vagotomy; 3, right vagotomy followed by left vagotomy; 4, cardiac sympathectomy and right cervical vagotomy, followed by left cervical vagotomy. Blood flow in the circumflex branch of the left coronary artery was observed by use of the thermostromuhr. Blood pressure was recorded optically from a cannulated femoral or carotid artery. The heart rate was observed electrocardiographically.

The effects of exercise on animals that had sympathectomized hearts were not essentially different from results obtained in animals that had innervated hearts. In both series exercise produced increased coronary blood flow, pulse rate and blood pressure. The observations were made 24 to 124 days after sympathetic ganglionectomy. The effects of exercise were very similar in animals on which complete cardiac denervation had been performed and those lacking only the vagi. Loss of the vagi affected cardiac acceleration profoundly. Vagotomized hearts increased only about 10 to 20 beats each minute with increments in the rate of work. This was true whether or not the sympathetic nerves were present. In the absence of marked acceleration and elevation of blood pressure the coronary blood flow was not affected by exercise. In animals that had vagotomized or totally denervated hearts the coronary blood flow appeared to be influenced chiefly by the blood pressure.

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THE DISAPPEARANCE OF T-1824 AND STRUCTURALLY RELATED DYES FROM THE BLOOD STREAM

MAGNUS I. GREGERSEN AND RUTH A. RAWSON

From the Department of Physiology, College of Physicians and Surgeons, Columbia University

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The rate at which vital dyes escape from the blood stream has for several years been of practical interest in connection with the determination of plasma volume with the dye method. Although observations have been made on a large variety of dyes (Dawson, Evans and Whipple, 1920; and others), little has been accomplished to explain the remarkable ability of certain dyes to leave the blood slowly (notably T-1824 and brilliant vital red) or to account for the striking differences in rate of disappearance exhibited by dyes of similar structure and molecular weight. These questions have an important bearing on the interpretation of experiments in which dyes are employed for measuring the plasma volume or for demonstrating local or overall changes in the permeability of the vascular bed. The present investigation concerns the behavior of four dyes—T-1824, trypan blue, niagara sky blue and niagara sky blue 6B—and the interpretation of their behavior in the light of recent electrophoretic studies (Rawson, 1943).

EXPERIMENTAL PROCEDURE. Commercial batches of trypan blue have long been known to contain considerable amounts of a red component which has been found to be more diffusible than trypan blue itself (von Möllendorf, 1914). Impure samples would therefore be unsuitable for the present studies and give an erroneous impression of the rate at which the dye leaves the blood stream. For these studies the dye was purified[1] until the "capillary test" showed that all traces of the red impurities had been removed. By the same token the other three dyes were known to be free of contaminating isomers.

Four adult mongrel dogs, one male and three females, accustomed to the procedure of blood volume determinations, were selected for the tests. They were given water *ad libitum* but no food for twenty hours before each experiment. The tests were carried out with the dog resting quietly on an animal board. After withdrawal of a dye-free sample of blood from one of the jugular veins, a measured amount of the dye to be tested was injected into this vein with a calibrated syringe. Samples (2 cc.) were then collected without stasis from the opposite jugular vein at exactly 5, 10, 15, 20, 30, 40, 50, 65, 80 and 95 minutes after the injection. The dye determinations were made on the serum with a Koenig-Martens visual spectrophotometer, each dye being read at the wave

[1] The dyestuff is precipitated 4–5 times with sodium acetate to remove the alcohol insoluble salts. The final precipitate is then dissolved in a small volume of hot water and immediately poured into several volumes of alcohol. The dye forms a fine precipitate which settles out, whereas the sodium acetate and "red component" remain in solution. The alcohol precipitation is repeated until the "capillary test" is negative for red.

length where its spectral absorption in serum is maximal (Gregersen and Gibson, 1937). For injection the dyes were made up in 0.5 per cent solution in distilled water. These solutions were standardized by determining the optical density at a dilution of 1:250 in serum. The values are given in table 1.

RESULTS. In order to assemble the dye curves for direct comparison and to calculate in each instance the fraction of dye which has escaped or been removed from the plasma over a given period after the injection it becomes necessary to express the plasma dye concentrations in terms of an initial concentration. We shall use the term *initial concentration* here to mean the concentration that obtains if 100 per cent of the dye is uniformly mixed with the circulating plasma. This value is obviously not directly measurable because of the inescapable fact that by the time the dye is uniformly distributed some of it has already left the bloodstream. The time required for mixing and the amount of dye lost during this period must therefore be considered in estimating the initial concentration.

TABLE 1

DYE	WAVE LENGTH	OPTICAL DENSITY 0.5%—1:250 IN SERUM
	mμ	
T-1824*	620	1.65
Niagara sky blue 6B	620	1.34
Trypan blue	605	1.41
Niagara sky blue	600	0.95

* Supplied by Warner Institute of Therapeutic Research.

From a consideration of the speed of the circulation of the blood it has been generally inferred that mixing would be effected in from 2 to 5 minutes (Erlanger, 1921). This conclusion is apparently supported by the fact that within 2 to 3 minutes after the injection of dye there is no longer a measurable difference in dye concentration among samples taken simultaneously from various regions of the body (Gilder, Müller and Phillips, 1940). It is to be noted, however, that this test does not prove that the dye has been mixed with all of the plasma in the vascular bed.

Time-concentration curves have been variously interpreted. Keith, Rowntree and Geraghty (1915) who introduced the dye method considered that mixing was complete in 4 to 6 minutes. Subsequent studies with more precise methods of measuring dye concentration have indicated that the mixing time may be considerably longer (Gregersen et al., 1935–39; Gibson and Evans, 1937; Price and Longmire, 1942). Robinow and Hamilton (1940) lean in the opposite direction, stating that "the shape of the disappearance curve suggests . . . that mixing of the dye is complete after two or three complete circulations." Hahn and his associates (1942) find that the tagged erythrocytes mix completely in 2 to 4 minutes with all the red cells and with about four-fifths of the plasma. Hahn puts forth the proposition that the remaining one-fifth of the plasma is present largely as a stagnant or slowly moving peripheral film lining the walls of the small vessels and capillaries and that dye reaches this film slowly by diffusion from the axial stream. His estimate of the amount of plasma which is slowly mixed seems rather high. In the first place the change in dye concentration (T-1824) during what Hahn terms the "second phase" of mixing is seldom greater than 5 to 10 per cent. In the second place it is probable that only about 10 per cent of all the blood is present in the capillary bed (Bazett, 1941) and of this a considerable fraction must be in active circulation. The diffusion of dye from the axial stream into the

peripheral films may possibly be a factor during the second phase of mixing, but the phenomenon of "vasomotion" (Chambers, 1942) and the intermittent capillary flow (Fulton and Lutz, 1940) would seem to be more important.

TABLE 2

DOG NO.	SEX	B.WT.	DYE	LINEAR PLOT (OPTICAL DENSITY VS. TIME)				LOG PLOT (LOG OF OPTICAL DENSITY VS. TIME)		SQUARE ROOT PLOT (OPTICAL DENSITY VS. $\sqrt{t}$)	
				Optical density		Dye lost in 60 min.	Plasma volume	Opt. dens. 0 min. (by extrapolation)	Plasma volume	Opt. dens. 0 min. (by extrapolation)	Plasma volume
				0 min. (by extrapolation)	60 min.						
Date: 7-9-41. T-1824											
		kgm.	*cc.*			*per cent*	*cc.*	*cc. kgm.*		*cc.*		*cc.*
1	F	13.90	4.06	1.37	1.28	6.5	568	40.8	1.37	568	1.40	556
2	M	11.77	4.07	1.24	1.11	10.4	631	53.7	1.25	621	1.305	605
3	F	10.84	3.08	0.915	0.82	10.3	637	58.8	0.915	637	0.975	598
4	F	10.20	3.07	1.01	0.935	7.5	584	57.7	1.01	584	1.12	526
Averages...		11.68				8.8	605	52.7		603		571
Date: 6-19-41. Niagara Sky Blue 6B (D-1824)												
1	F	13.35	5.03	1.53	1.35	11.7	512	38.4	1.535	510	1.58	495
2	M	12.02	5.05	1.30	1.06	18.4	604	50.2	1.30	604	1.365	575
3	F	11.37	5.05	1.14	0.98	14.0	688	60.5	1.14	688	1.19	659
4	F	10.40	4.06	1.105	0.93	15.8	573	56.1	1.105	573	1.11	570
Averages...		11.79				15.0	594	51.3		594		575
Date: 6-26-41. Trypan Blue (T-1836)												
1	F	13.51	5.03	1.44	0.86	40.3	582	43.0	1.48	567	1.51	555
2	M	12.34	5.05	2.42	1.35	44.2	736	59.6	2.45	727	2.54	701
3	F	10.71	5.05	1.16	0.67	42.2	711	66.4	1.18	699	1.25	660
4	F	10.07	4.06	1.04	0.68	34.6	640	63.5	1.05	634	1.11	600
Averages...		11.66				40.3	667	58.1		657		629
Date: 6-14-41. Niagara Sky Blue (D-1836)												
1	F	13.06	5.03	2.20	1.37	37.7*	601	46.1	2.2	601	2.345	563
2	M	12.28	5.05	2.00	0.93	53.5	663	54.0	2.0	663	2.2	603
3	F	11.24	5.05	1.90	0.85	55.3	698	62.0	1.9	698	1.96	676
4	F	10.19	4.06	1.80	0.85	52.7	594	58.3	1.82	587	1.82	587
Averages...		11.69				53.8	639	55.0		637		607

* Not included in average.

After a dye is uniformly mixed with the circulating plasma and until it re-enters the circulation by way of the lymph, the time-concentration curve presumably defines the rate at which the dye is passing out of the plasma compartment. From lymph studies with brilliant vital red (Smith, 1925) and with T-1824 (Ferrebee, Leigh and Berliner, 1941) it

may be concluded that re-entry of dye does not modify the disappearance curve perceptibly for at least an hour after the injection. The amount of dye returned to the blood stream through the thoracic duct during this time is only about 2 per cent of the total dye injected. Hence if one accepts the proposition that the curve from 10 minutes up to about one hour represents a continuous process which begins at the moment of injection, then the initial concentration should be obtainable by appropriate extrapolation of this curve to the zero time ordinate (time of injection). The values for the initial concentration (optical density at 0 min.) given in table 2 under the heading "Linear Plot" were obtained by simply extrapolating a smooth curve drawn through the points from 10 to 65 minutes. Other methods of making the extrapolation will be considered presently.

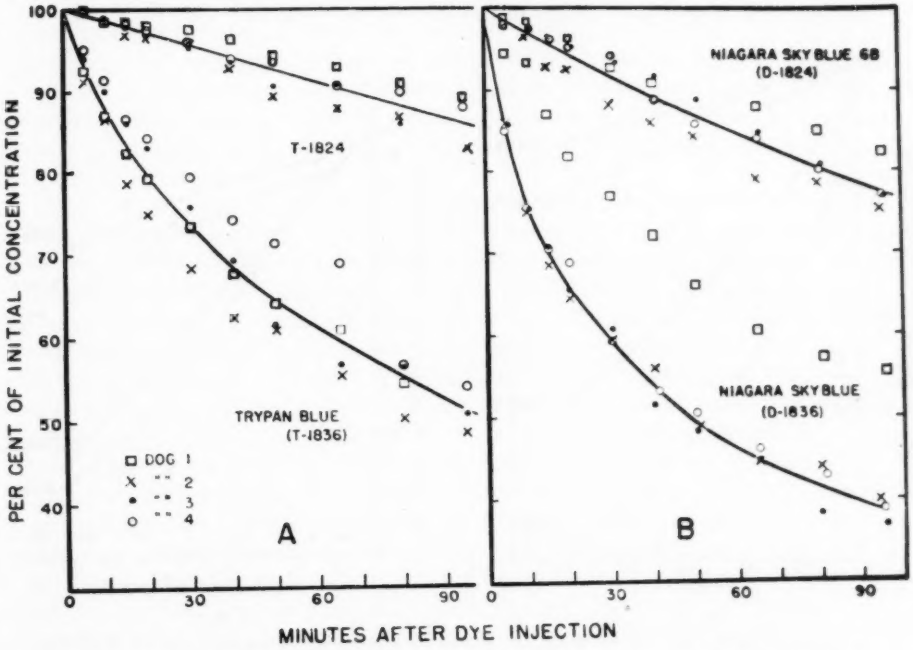

Fig. 1. Showing the rates of disappearance from the bloodstream of four structurally similar dyes. Each curve represents the average of four tests, except in the case of niagara sky blue where the results on dog 1 were excluded in calculating the average.

The graphs obtained by plotting the optical density determinations as per cent of the extrapolated initial value are shown in figure 1. It will be seen that with one exception, the test with niagara sky blue on dog 1, the results from dog to dog are quite consistent, showing clearly the differences in the escaping tendencies of the dyes. The heavy line drawn through the group of determinations with each dye has been plotted from the calculated averages of per cent dye remaining at various times after injection. Table 2 shows the per cent dye lost in 60 minutes in each test and the average loss for each dye. The value 8.8 per cent for T-1824 agrees closely with the average of several hundred determinations made on dogs in this laboratory during the past few years.

The time-concentration curve of each of the dyes under consideration here presumably follows some mathematical function which, if known, would greatly facilitate reliable extrapolation and derivation of the initial concentration. On a linear plot the disappearance curve of T-1824 up to one or two hours is often indistinguishable from a straight line (see fig. 1), and for the practical purpose of extrapolation to calculate the plasma volume it may be considered as such. That it is not actually a linear function can be demonstrated by exaggerating the vertical scale (concentration) or by compressing the horizontal scale (time). The curve then takes on the character of a logarithmic function, and indeed if the data are plotted as the logarithm of concentration against time the result is,

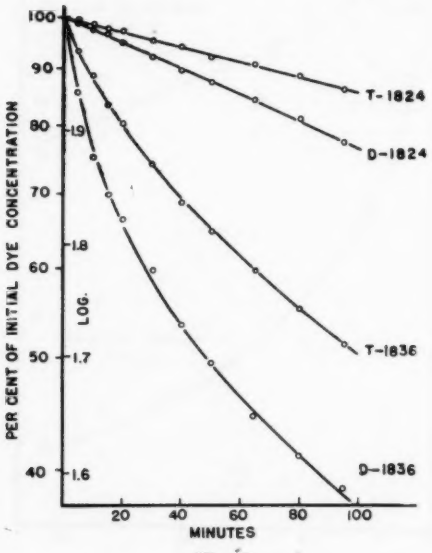

Fig. 2

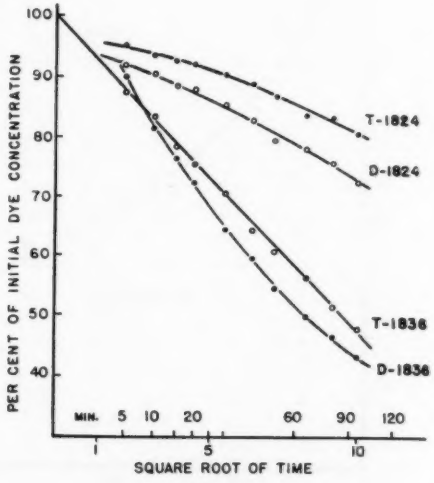

Fig. 3

Fig. 2. Semilog plot of the average values used in constructing the curves shown in figure 1.

Fig. 3. The same data with dye concentration plotted against the square root of time.

as far as one can tell, a straight line for a period of at least an hour after the injection (see fig. 2). Extrapolation gives initial values for T-1824 which are essentially identical with those obtained by extrapolation of the linear plot (see table 2). With niagara sky blue 6B, the semilog plot also gives a straight line and the extrapolated values agree with those from the linear plot. The method is however of little help in dealing with the data on trypan blue and niagara sky blue, for as may be seen in figure 2 the derivation of the initial concentration still involves the extrapolation of a curve.

The same data are shown in figure 3 plotted against the square root of time[2]

[2] The data were submitted to Dr. B. G. King and the values in table 2 are based on his decision as to where the line should be drawn through the points.

(King, Oppenheimer and Cole, 1943). As would be expected, the extrapolation of the curves gives higher values for the initial concentration than on the linear or semilog plot (see table 2).

A comparison of the plasma volumes determined with the four dyes (table 2) reveals differences which do not represent actual changes in volume. The determinations with T-1824 and niagara sky blue 6B give essentially the same average values for the group (605 and 594 cc.), whereas those obtained with trypan blue and niagara sky blue are notably higher (667 and 639 cc.). The discrepancy between the plasma volumes as determined with trypan blue and T-1824 was subsequently confirmed by running both tests on the same dog within a period of three hours. In two such experiments on dogs 1 and 4 the plasma volumes with trypan blue were respectively 568 and 735 cc. The values obtained 2 to 3 hours later with T-1824 were 506 and 667 cc. respectively. (The per cent trypan blue lost during the first hour after injection was 43.2 in dog 1 and 40.9 in dog 4.) The reasons for these discrepancies will be considered in the following discussion.

Discussion. If the observations presented above are examined in relation to the chemical structure of the four dyes it becomes evident that we are dealing here with two modifications in structure which independently influence the escaping tendency of the dyestuff. It will be noted that trypan blue and T-1824 are tolidine dyes, whereas niagara sky blue and niagara sky blue 6B are dianisidine dyes. The only other structural difference among the dyes is in the position of the sulphonic acid radicals on the naphthalene rings. The structural relationships are at once apparent if one adopts a descriptive terminology to conform with the name T-1824. Thus trypan blue may be designated as T-1836, niagara sky blue as D-1836, and niagara sky blue 6B as D-1824.

The results included in figure 1 and table 2 show that a shift in the sulphonic acid radicals from the 2–4 to the 3–6 positions profoundly alters the disappearance rate. In the case of the two tolidine dyes this modification in structure increases the fraction of dye lost in the first hour from an average of about 9 per cent to 40 per cent (cf. T-1824 and T-1836, fig. 1A). In the case of the dianisidine dyes (cf. D-1824 and D-1836, fig. 1B), the same change in structure raises the fraction lost from 15 per cent to 54 per cent. Furthermore, a change from the tolidine to the dianisidine structure also increases the disappearance rate. With the 1824 form of the naphthalene ring this change increases the loss of dye in the first hour from 8 per cent to 15 (cf. T-1824 and D-1824, fig. 1), and with the 1836 naphthalene ring it raises the loss from 40 per cent to 54 (cf. T-1836 and D-1836, fig. 1).

What is the explanation of these differences in the escaping tendency of dyes so closely related structurally? A number of facts concerning the behavior of well-known vital dyes lead one to suspect that they combine with the plasma proteins. There is a good deal of indirect evidence that this is true, for example, of T-1824. This dye leaves the blood stream at approximately the same rate as antibody globulin (Culbertson, 1934). Furthermore, at plasma levels required for the determination of plasma volume T-1824 is not found in the urine unless the latter also contains protein. It is also known that protein stabilizes

the spectral absorption curves of T-1824 and increases the solubility of the dye in sodium chloride (Gregersen and Gibson, 1937). The fact that T-1824 is not removed from plasma by precipitating the fibrinogen with heat and that all the dye in whole blood is recovered in the serum after normal clotting has taken place (Gregersen and Schiro, 1938) would appear to exclude fibrinogen from any part in the binding of the dye in the blood stream.

Direct evidence for the binding of various dyes by protein has been obtained by Rawson (1943) with the electrophoretic technique of Tiselius. She found that not only T-1824 but also T-1836, D-1824 and D-1836 combine entirely with the albumin fraction when these dyes are mixed with plasma or serum. She also found that the dye-albumin complexes differed strikingly in their tendency to dissociate when exposed to a cellophane surface, as shown by the staining of the cellophane. In the case of T-1824 in serum, none of the dye was transferred to the cellophane after 24 hours. D-1824 stained the strip slightly, whereas T-1836 and especially D-1836 left the strip deeply stained. Comparison of these observations with the time-concentration curves in figure 1 suggests that the differences in the rates at which the dyes leave the circulation are determined by the differences in the strengths of the bonds which the dyes form with the albumin. Furthermore it would appear that in the case of the dye T-1824 the binding is essentially equivalent to a tagging of the albumin.

According to Smith (1925) who carried out extensive investigations with brilliant vital red, a dye which in its behavior closely resembles T-1824, the process of elimination involves 1, diffusion from the plasma; 2, temporary storage in phagocytes and reticulo-endothelial system, and finally 3, excretion of the dye by the liver. The final process, excretion by the liver, must of course sever the dye-protein bond. Does this occur also during phagocytosis? Information concerning the mechanism by which the dye-albumin bond is severed in the body would be extremely desirable. The fact that Smith found a striking similarity in the dye and protein content of lymph from various regions may be regarded as evidence that the dye-protein bond is still intact in the tissue fluids and in the lymph.

One problem which must be reconsidered in the light of the preceding evidence is that of choosing among the linear, semilog and square root plots for determining the initial concentration of T-1824 by extrapolation. The test by plotting is unfortunately not always critical because of experimental variations in the data. That these variations need not be great in order to leave the issue in doubt may be readily seen by comparing the results of plotting a theoretical time-concentration curve in the three ways as shown in figure 4.

A straight line was arbitrarily drawn on the semilog plot in figure 4B to represent a loss of 15 per cent of the dye in 60 minutes. Values taken from this line are shown as hollow circles on the linear and square root plots in figures 4A and 4C. It will be noted that the linear plot of these data is so nearly a straight line that extrapolation on that assumption gives an initial concentration which is practically the same as that shown in the original semilog plot. On the square root plot, however, the values lie on a distinct curve. But one thing

must be noted, that if one takes into account only the points from 10 minutes to 90 minutes the deviation from a straight line is not great. Hence if one were dealing with actual observations in which the 10-minute value was slightly high because of incomplete mixing the plotted data might possibly be interpreted as a straight line on a square root plot. However the adoption of this interpretation as suggested by King, Cole and Oppenheimer (1943) implies that dye is lost rapidly during the mixing period, the rate approaching infinity at the time of

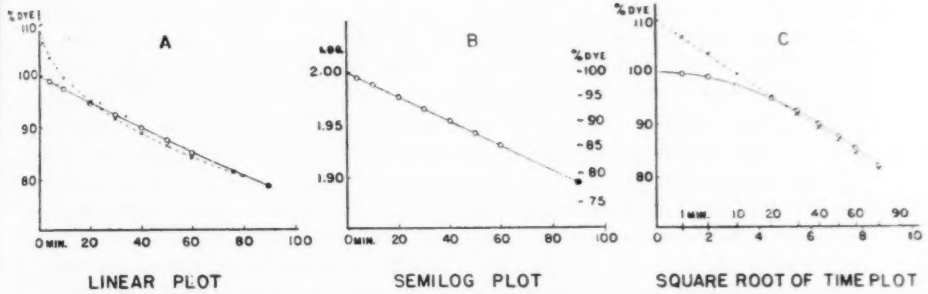

Fig. 4. Showing the curves obtained by transposing values from a straight line on a semilog plot (B) to a square root plot (C) and a linear plot (A). It will be noted that the *initial concentration* obtained by extrapolating the curve in (A) on the assumption that it is a straight line would differ only slightly from 100 per cent, whereas "straight line extrapolation" of (C) gives a value of 110 per cent.

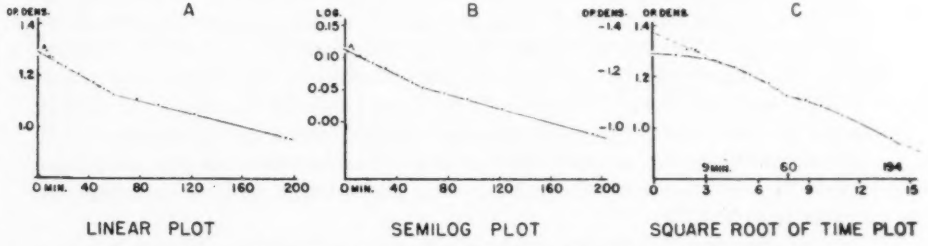

Fig. 5. Linear (A), semilog (B) and square root of time (C) plots of a typical time-concentration curve of T-1824 in a normal dog. "Straight line extrapolation" in (A) gives an initial concentration of 1.29; in (B) 1.30; in (C), 1.38.

injection (see broken line in fig. 4A). There is no valid evidence of such a rapid initial loss of T-1824.

From a critical examination of various data on semilog and square root plots we have concluded that during the first hour after injection the time-concentration curve is more precisely described by a logarithmic function than by a square root function. An illustration is given in figure 5. Both the linear and semilog plots of these data apparently give a straight line from 6 to 60 minutes after the injection, whereas the square root of time plot during the same interval is slightly curved. It should be pointed out also that in the square root plot of

the average curve for T-1824 in figure 3 there is a suggestion of a similar curvature which does not appear in the semilog plot of the same data in figure 2.

The logarithmic character of the disappearance curve of T-1824 is also in accord with the fact that this dye is firmly bound to the albumin. During the time when the disappearance rate is being determined, both the plasma volume and the plasma protein concentration presumably remain unchanged. Under these conditions the albumin must escape from and be returned to the circulating plasma at a constant rate. If the dye leaves the circulation only in combination with the albumin, then it follows that the amount of dye which escapes in unit time must be a constant fraction of the total amount of dye remaining in the blood. This is the basic condition for an exponential disappearance function and therefore the time-concentration curve should give a straight line on a semilog plot. That it does so seems to be shown by figures 2 and 5. However, when the dye begins to re-enter the circulation by way of the lymph, the same logarithmic function could not be expected to fit the data. Time-concentration curves of T-1824 on normal dogs regularly show a break. In the curve shown in figure 5 the break occurred at one hour, but as a rule it appears about two hours after the injection of the dye. Similar conclusions regarding the time-concentration curve have been reached by Hemingway, Scott and Wright (1935) in a study with the dye, water blue, and by Price and Longmire (1942) with T-1824.

The question may be raised as to why the time-concentration curves of trypan blue and niagara sky blue do not also follow a semilog function (see fig. 2). This clearly means that the mechanism by which these dyes escape from the plasma is not the same as for T-1824, and indeed certain facts already mentioned bear this out. The cellophane staining test (Rawson, 1943) shows that trypan blue and niagara sky blue are not as firmly bound to the plasma albumin as T-1824, and one would therefore expect that vital staining might be a large factor in their removal from the plasma. Initial loss of dye by staining probably accounts for the fact that these dyes give higher values for plasma volume (table 2). Furthermore, they escape into the urine, a path of excretion not available to T-1824.

It may be noted finally that one implication of the preceding discussion is that the rate of disappearance of T-1824 during the first hour after the injection is a measure of the rate of exchange of albumin. If this be true, the dye is obviously a convenient tool for studying changes in the local or overall "permeability" of the vascular bed to albumin.

CONCLUSIONS

From a correlation of the disappearance rates of T-1824, T-1836 (trypan blue), D-1824 (niagara sky blue 6B), and D-1836 (niagara sky blue) with Rawson's (1942) studies of dye-protein binding, it is concluded that the difference in disappearance of these dyes from the bloodstream is determined by the strength of the bond which is formed between the dye and plasma albumin. Although the dyes are closely related structurally they exhibit remarkable differences in behav-

ior (see fig. 1 and table 2) which are clearly related to their differences in structure (see Discussion).

The various lines of evidence which are cited suggest that T-1824 is so firmly bound to the albumin that the disappearance rate of this dye during the first hour after injection is a measure of the rate of escape of the circulating albumin. The disappearance curve of the dye should then follow a semilog function. This prediction is in our opinion confirmed by a critical examination of time-concentration curves obtained on normal dogs (figs. 2 and 5).

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THE BINDING OF T-1824 AND STRUCTURALLY RELATED DIAZO DYES BY THE PLASMA PROTEINS

RUTH A. RAWSON[1]

From the Department of Physiology of the College of Physicians and Surgeons, Columbia University, New York City

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The purpose of the present study was to find out if the differences in disappearance rates of T-1824, trypan blue, niagara sky blue and niagara sky blue 6B depend upon the binding of these dyes by the plasma proteins (Gregersen and Rawson, 1943). The affinity of the various dyes for the plasma proteins was investigated: 1, with the electrophoresis method of Tiselius (1937); 2, by the ultracentrifuge; 3, by the effects of the plasma proteins upon the spectral absorption of the dyes, and 4, by a cellophane-staining test.

ELECTROPHORESIS EXPERIMENTS. The protein solutions were prepared for electrophoresis as follows: 4 cc. of serum or plasma were diluted with 12 cc. of a 0.2 M phosphate buffer, varying in pH from 7.40 to 7.60, and containing 0.15 M sodium chloride. The mixture was dialyzed at 5°C. in a cellophane bag for two or more days against two liters of the buffer. The buffer solution was changed at least once during the dialysis period. The dye was added from stock solutions before dialysis.[2]

Figure 1a shows the electrophoretic pattern of normal citrated human plasma. The pattern in figure 1b is produced by the same plasma containing T-1824 at a concentration of 0.004 per cent. The light absorption of the dye causes a well-defined shaded area. The fact that the shading begins with the ascending albumin boundary and ends with the descending albumin boundary demonstrates that the dye migrates entirely with the albumin. The same results were obtained with trypan blue, niagara sky blue, niagara sky blue 6B and also with brilliant vital red. In dog serum, as in human plasma, all of the dyes also migrated entirely with the albumin fraction. At the close of each dye-plasma experiment the protein boundaries were pushed by clockwork and plunger in order to separate the alpha, beta and gamma globulins on the descending side from the rest of the solution. Chemical and spectrophotometric examination of the globulin solution showed the presence of protein and the absence of dye.

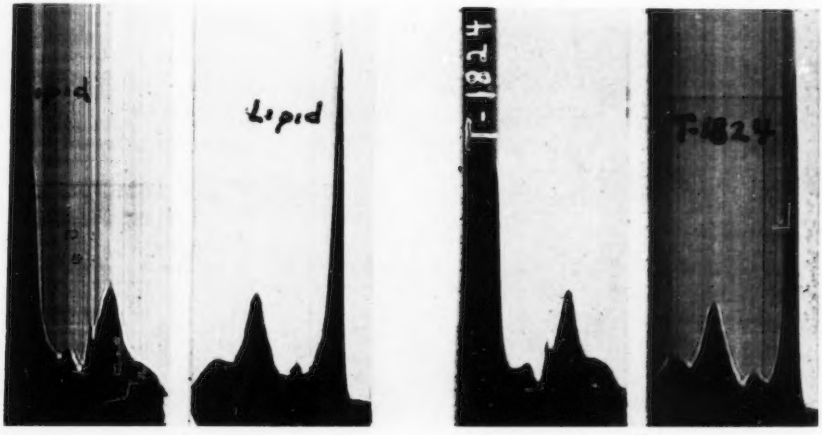
The electrophoretic pattern of human plasma containing 0.098 per cent T-1824 (after dialysis) showed the dye boundary to begin with the ascending albumin boundary and to end with the descending beta globulin boundary. Hence if sufficient dye is present it will be bound by the alpha and beta globulins as well as by the serum albumin. The descending gamma globulin was free of dye. At the end of four hours of electrophoresis, the fractions listed in table 1 were

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

[2] Since trypan blue and niagara sky blue stained the cellophane bags during dialysis it was necessary to add these dyes to the plasma samples after dialysis.

separated. The concentrations of dye and protein in the different fractions were obtained by the König-Martens spectrophotometer and micro-Kjeldahl determinations. It should be noted that the ratio of T-1824 to albumin in the albumin fraction was approximately eight moles of dye per mole of albumin.

Experiments with T-1824 and electrophoretically separated human serum albumin. T-1824 was added to a sample of electrophoretically separated human serum albumin in high concentration. The final concentration of dye (after



1*a* 1*b*

Fig. 1. Electrophoretic pattern of normal human plasma. *a*, without, and *b* with T-1824 added.[3]

TABLE 1

The distribution of T-1824 (0.098 per cent) in human plasma after electrophoresis

ELECTROPHORETIC SAMPLE	PROTEINS PRESENT	PER CENT DYE*	MGM. N PER CC.	MOLES OF DYE† / MOLES OF PROTEIN
1: Before electrophoresis	Albumin and globulins	0.098	1.74	
2	Albumin	0.063	0.76	8.3
3	Globulins	0.006	0.41	5.0
4	Gamma globulins	0.00	0.086	

* See page 710 for method of calculating dye concentration.

† Molecular weights used in calculating ratio: Albumin 70,000, Globulin 150,000, Dye 960.

dialysis) was 0.019 per cent in 0.098 per cent albumin, the ratio of dye to albumin being fourteen moles of dye per mole of albumin. After four hours the T-1824 migrated ahead of the albumin on the ascending side. Table 2 gives the mobility of the albumin before and after the adding of T-1824. The mobility of albumin is not affected by T-1824 at a concentration of 0.004 per cent (ratio of

[3] According to Blix, Tiselius, and Svensson (1941) the shading beginning and ending with the beta globulin boundaries is caused by the presence in the plasma of the lipids which migrate with the beta globulin.

dye to albumin, 1 to 28) but if the dye concentration is increased to 0.019 per cent (ratio of dye to albumin, 14 to 1) the mobility is increased from 5.0×10^{-5} to 7.0×10^{-5}. The significance of this change in mobility will be discussed later.

Electrophoresis of T-1824 in globulin solution. A 2.3 per cent globulin solution prepared from normal human plasma by the method of Howe (1921) showed the presence of two protein fractions corresponding in mobilities to alpha and gamma globulin. When T-1824 was added to this globulin solution to a concentration of 0.002 per cent, the dye migrated wholly with the alpha globulin.

ULTRACENTRIFUGE AND DIFFUSION EXPERIMENTS. The observation that the dyes migrate preferentially with the albumin fraction has been confirmed by the sedimentation of T-1824 with serum albumin in the ultracentrifuge. The molecular weights of the albumin-dye complexes in solutions of various dye-al-

TABLE 2

The effect of a high concentration of T-1824 on the mobility of human serum albumin

	CONCENTRATION OF		MOLES OF DYE / MOLES OF ALBUMIN	pH	MOBILITY OF ALBUMIN ($\mu \times 10^5$)	
	Albumin mgm/cc. (7.0 × N)	Dye per cent			A*	D*
Serum albumin	0.98	no dye		7.45	5.00	5.50
Serum albumin plus high concentration of T-1824	0.98	0.019	14	7.48	7.03	7.65

* A is the mobility of the ascending boundary, D the mobility of the descending boundary.

bumin ratios were calculated from the sedimentation rates and diffusion constants according to the formula of Svedberg (Svedberg and Pedersen, 1940).

$$M = \frac{RTS}{D(1 - Vp)}$$

where $R = 8.212 \times 10^{-7}$

$T = 293°$ absolute
$D =$ Diffusion constant at 20°C.
$S =$ Sedimentation rate at 20°C.

The sedimentation rates were determined in an air driven ultracentrifuge at 800 revolutions per second (158,000 × gravity) and at 24° to 31°C. The diffusion constants were determined at 2° or 8°C. in an electrophoresis cell and diffusion curves were obtained at intervals up to 75 hours on photographic plates using the Longsworth schlieren scanning method (1939). The sedimentation rate and diffusion constant of electrophoretically separated human serum albumin in phosphate buffer (pH 7.49) were determined under the following conditions: 1, without dye; 2, containing 0.00049 per cent T-1824, and 3, containing 0.038 per cent T-1824 (see table 3).

In each of the three sedimentation experiments only one boundary was present. When dye was present it sedimented with the albumin leaving the supernatant buffer colorless. Table 3 lists the data obtained. It should be noted that in a preliminary experiment in which T-1824 in 0.004 per cent concentration in phosphate buffer was centrifuged at 900 revolutions per second, no sedimentation was observed after 1½ hours.

Discussion of electrophoresis and ultracentrifuge data. The binding of the dyes by the plasma proteins is clearly demonstrated in the electrophoresis experiments (fig. 1). This is further borne out in the case of T-1824 by the ultracentrifuge studies. The dyes, T-1824, niagara sky blue 6B, trypan blue, niagara sky blue and brilliant vital red in 0.004 per cent concentration in plasma are wholly and preferentially bound by the albumin fraction.

The experiments show that if the dye concentration is increased sufficiently, the acid dyes may also be bound by the globulin fraction. Furthermore when

TABLE 3

*The molecular weight of human serum albumin before and after adding T-1824 as determined in the ultracentrifuge**

	CONCENTRATION OF		MOLES OF DYE / MOLES OF ALBUMIN	DIFFUSION CONSTANT AT 20°C. × 10^7	SEDIMENTATION RATE AT 20°C. × 10^{13}	MOLECULAR WEIGHT
	Dye per cent	Albumin mgm/cc. (N × 7.0)				
Serum albumin	0	5.60	0.0	6.3	4.56	70,300
Serum albumin plus dilute T-1824	0.00049	5.60	0.067	5.9	4.32	71,000
Serum albumin plus concentrated T-1824	0.038	4.97	4.8	6.0	5.13	83,000

* The serum albumin used in these experiments was electrophoretically separated from human plasma.

T-1824 is added to a solution of alpha and gamma globulins it migrates preferentially with the alpha globulin.

The data make it possible to estimate the approximate number of molecules of T-1824 which can be bound by a molecule of albumin at physiological pH and salt concentration. This dye in plasma migrates only with the albumin fraction unless the ratio of dye to albumin is increased beyond eight moles of dye per mole of albumin. When T-1824 is added to an albumin solution so that the ratio of dye to albumin is 14, some of the dye slowly leaves the albumin during electrophoresis. This would indicate that albumin can bind somewhat less than 14 molecules of dye per molecule of albumin. The ultracentrifuge data show that T-1824 is bound by the albumin in increasing proportions as the concentration of dye is increased, and this is also indicated in the electrophoresis experiments by the effect of T-1824 in low and high concentrations on the mobility of human albumin (table 2). The increase in mobility of human albumin containing relatively high concentrations of T-1824 at pH 7.4 may be explained by the assumption that as T-1824 is attached to the albumin it prevents the dissociation

of some of the basic groups. This shows that when a sufficient amount of dye is bound to albumin the isoelectric point of the albumin-dye complex is significantly lower than that of albumin.

ABSORPTION SPECTRA OF THE DYES IN PROTEIN SOLUTIONS. The absorption spectra for the dyes (T-1824, niagara sky blue 6B, trypan blue and niagara sky blue) were determined by Gregersen and Gibson (1937) in water, 0.9 per cent sodium chloride and plasma. The curves of T-1824 and niagara sky blue 6B in plasma show an asymmetry about the point of maximum absorption which is not present in the water solutions. This suggests that the effect of albumin and globulin on the absorption spectra of these dyes might be related to the binding of these dyes by the proteins.

The observations were made with a König-Martens visual spectrophotometer. The albumin solutions prepared by ammonium sulfate precipitations were found by electrophoretic analysis to be free of globulin. Howe's method (1921) for preparing the globulin solution was used, and according to electrophoretic analysis, this solution contained only alpha and gamma globulins. The protein solutions were dialyzed (5°C.) for three days against running water, and for two days with several changes of distilled water or phosphate buffer (pH 7.40).

Figure 2 shows the absorption curves of the four blue dyes in human plasma, human albumin and human globulin solutions. In each instance the shape of the curve obtained in albumin solutions (1.0 per cent) was the same as that in plasma. Globulin solutions in concentrations as great as those occurring in plasma depressed the optical density and shifted the maxima of the dyes from that observed with buffer (Gregersen and Gibson, 1937), but did not produce the asymmetry as seen in the plasma curves of T-1824 and niagara sky blue 6B.

That the changes in the absorption spectra of the dyes produced by plasma are caused mainly by the albumin fraction is demonstrated by the results presented in figure 2. The effect of various concentrations of electrophoretically pure albumin were therefore studied in greater detail and the results are given in figure 3. Figure 4 shows the change of optical density at the maximum for the four dyes as plotted against the ratio, moles of albumin per mole of dye.

Comparison of the optical density at the point of maximum absorption of solutions of 0.001 per cent and 0.002 per cent T-1824 in various concentrations of albumin showed that the Lambert-Beer Law holds in this range of concentration when protein-dye solutions of the same ratio of protein to dye are compared. This was the range of concentration in which the optical density measurements were made.

From the above and figure 3 it is evident that within certain ranges of dye and albumin concentration the concentration of dye cannot be determined unless the ratio of dye to albumin is known. However, by plotting a full curve for the unknown solution (520 mμ to 640 mμ) and by matching this curve in respect to *both shape and point of maximum absorption* with those obtained on solutions of known dye-albumin concentration, and relating the optical density at the point of maximum absorption as in the equation below, the true concentration of dye in the unknown can be determined.

$$\text{Per cent concentration of dye in unknown} = \frac{\text{O.D. of unknown}}{\text{O.D. of the 0.002 per cent curve}} \times 0.002 \text{ per cent}$$

A check on the ratio of dye to protein obtained by this method was made by a micro-Kjeldahl determination of the protein concentration and in all experiments the two values checked quantitatively.

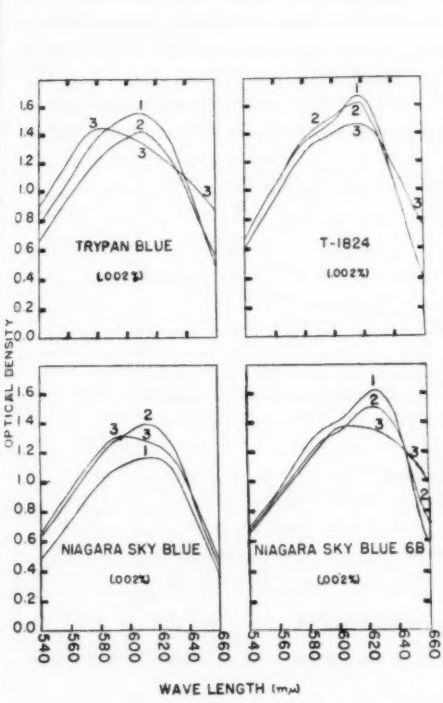


Fig. 2

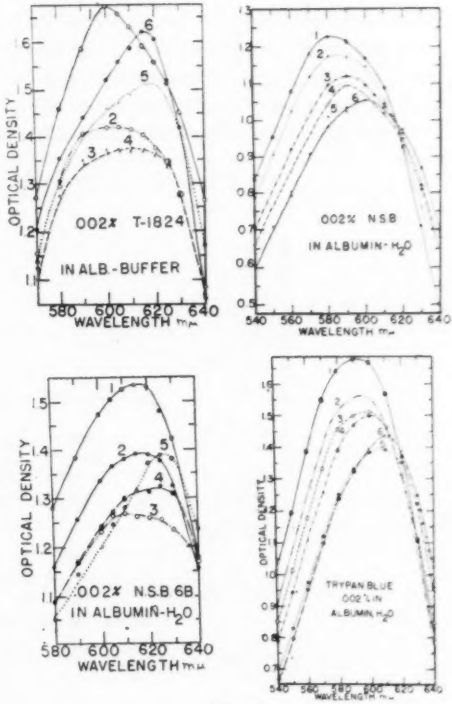


Fig. 3

Fig. 2. Absorption spectra of the dyes in 1, plasma; 2, plasma albumin, and 3, plasma globulin.

Fig. 3. The effect of varying the albumin concentration on the absorption spectra of the four dyes (0.002 per cent). The curves for T-1824 were obtained in phosphate buffer at pH 7.3; the curves for the other three dyes were obtained in water. The moles of albumin per mole of dye were as follows: T-1824 (1) 0.000 (2) 0.057 (3) 0.096 (4) 0.11 (5) 0.48 (6) 4.8; niagara sky blue 6B (1) 0.00 (2) 0.099 (3) 0.14 (4) 0.20 (5) 0.99; trypan blue (1) 0.00 (2) 0.053 (3) 0.096 (4) 0.24 (5) 0.48 (6) 7.7; niagara sky blue (1) 0.00 (2) 0.10 (3) 0.14 (4) 0.20 (5) 0.50 (6) 7.9.

Discussion of spectral absorption experiments. The observations that the changes in the absorption curves of the four blue dyes produced by plasma are caused mainly by the albumin fraction (fig. 2) is in agreement with the preferential binding of these dyes by the albumin as observed in the electrophoresis studies. It should be noted that Robinson and Hogden (1941) have recently found that the albumin fraction of serum is also mainly responsible for the

changes produced by a serum-buffer system on the absorption spectrum of phenol red.

Definite differences were observed among the four dyes when the effect of various albumin concentrations on the absorption spectra were compared (figs. 3 and 4). A shift of about 20 mμ to the red end of the spectrum was observed with all the dyes in albumin solutions. This would indicate a dampening of the bond energies of the dye as binding with protein takes place. As the albumin concentration was increased in the presence of a constant amount of dye (0.002 per cent) the optical density at the point of maximum absorption of all four dyes fell (fig. 4). The optical density of T-1824 fell to a minimum value when the ratio moles of dye per moles of albumin was 11. The optical density rose when the concentration of albumin was increased and at high albumin concentrations

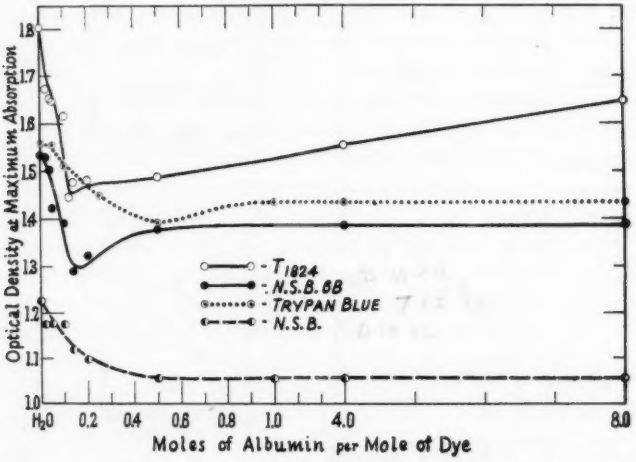


Fig. 4. Variation in optical density at maximum absorption with increases in albumin—dye ratio.

reached that observed in water. A similar variation in optical density was obtained with niagara sky blue 6B. The optical density fell until the ratio, moles of dye per moles of albumin was 8.3 and then increased in value. The rise in optical density from the point of maximum depression was small in trypan blue (fig. 4). Efskind (1940) has observed a similar result for trypan blue using dog plasma. No rise was observed with niagara sky blue.

The experiments suggest that the depression of the optical density by albumin represents binding of the dye, and the continuous fall in optical density with increasing protein concentration represents the conversion of free dye to dye-protein.[4] According to this concept the minimum value of the optical density

[4] The depression in optical density produced by albumin cannot be explained as a salt effect since Gregersen and Gibson (1937) found that sodium chloride produced no depression of the optical density in plasma solutions.

should represent the point at which the maximum number of dyes are bound per molecule of albumin. This interpretation is supported by the electrophoretic studies with T-1824, in which it was observed that the maximum number of moles of dye that could be bound by a mole of albumin lay between 8 and 14. When the concentration of albumin is further increased, the optical density rises as the composition of the dye-protein complex changes from a high ratio of dye to protein to lower ratios. It should be noted that in plasma volume determinations the effect of changes in albumin concentration on the optical density of T-1824 does not have to be considered since the ratio of moles of albumin per moles of dye is greater than 10. This fact is also evident from figure 10 of Gregersen and Gibson (1937).

CELLOPHANE STAINING TEST. Water solutions of the dyes do not stain cellophane, but if a trace of salt is added to the system, the dyes are immediately and completely deposited on the cellophane. The dye so deposited cannot be removed by washing with water. When 0.002 per cent solutions of these dyes in plasma were placed in cellophane bags and left in the icebox dialyzing against buffer, it was found at the end of a month that the bags containing niagara sky blue and trypan blue were heavily stained and the respective plasma solutions colorless, while the T-1824 and niagara sky blue 6B bags remained unstained and the dyes were still in solution. These observations are striking in view of the fact that niagara sky blue and trypan blue escape from the circulation much more rapidly than T-1824 or niagara sky blue 6B (Gregersen and Rawson, 1943). Since the relative concentration of dye to protein was the same in all bags, and since the electrophoresis experiments have shown that the dyes in this concentration are wholly bound by the albumin, the above observation indicates a difference in the degree of dissociation of the various dye-protein complexes in the presence of a cellophane surface.

The staining of cellophane by the four blue dyes in various concentrations of human albumin was therefore studied more carefully. For each dye a series of dye-albumin solutions were prepared by adding 0.2 cc. of 0.04 per cent dye to 3.8 cc. of albumin giving final concentrations of albumin ranging from 0.17 to 4.20 grams per cent. A strip of cellophane (approximately 6 x 12 mm.) was immersed in each tube and observations were made at intervals up to twenty-four hours.

The albumin solutions used in these experiments had been dialyzed against 0.02 M phosphate buffer, pH 7.4, and contained 0.15 M sodium chloride. The pH of the dye-protein solutions lay between 7.3 and 7.4 as determined by the glass electrode. An abbreviated protocol of the experiments with the four blue dyes in albumin solutions is given in table 4.

From the protocol, table 4, it is apparent that there is a difference in the affinities of the dyes for albumin in the presence of cellophane. Niagara sky blue-albumin solutions stained the cellophane strips within 1 to 2 hours even in tubes where the ratio of albumin to dye was 3 to 1. T-1824, in solutions of albumin having a ratio of one or more molecules of albumin to one of dye, did not stain cellophane strips in a period of twenty-four hours. The niagara sky

blue 6B albumin solutions stained the cellophane to only a slightly greater extent than the T-1824 albumin solutions. Deeper staining was observed with trypan blue-albumin solutions and the deepest staining occurred with niagara sky blue.

DISCUSSION. The progressive difference in the affinities of the four blue dyes for albumin in the presence of cellophane is apparent from these tests. Since the electrophoresis experiments have shown that these dyes, in the concentrations employed in the disappearance studies, are wholly bound by the albumin fraction of plasma these differences in their affinities for albumin are significant. It will be noted that the relative affinities of the dyes for albumin bear an inverse relation to their disappearance rates from the circulation. T-1824 has the highest affinity and the lowest disappearance rate (8 to 10 per cent per hour), while niagara sky blue has the lowest affinity and the highest disappearance rate (54 per cent per hour) as shown by Gregersen and Rawson (1943).

TABLE 4

Protocol of cellophane staining test

ALBUMIN CONCENTRATION (N × 7.0)	MOLES OF ALBUMIN PER MOLE OF DYE	T-1824 (0.002 per cent)		N.S.B.6B (0.002 per cent)		Trypan blue (0.002 per cent)		N.S.B. (0.002 per cent)	
		Time in hours							
		4	24	4	24	4	24	4	24
mgm./cc.									
4.20	2.9	−	−	−	−	−	+++	+++	++++
1.40	0.96	−	−	−	+	+	++++	+++	++++
0.70	0.48	−	±	−	++	+++	++++	+++	++++
0.28	0.19	−	++	−	++	++++	++++	++++	++++
0.20	0.13	−	++	±	+++	++++	++++	++++	++++
0.17	0.12	−	+++	±	+++	++++	++++	++++	++++

−, No staining; ±, cellophane same shade as solution; +, cellophane darker than solution.

NSB6B = niagara sky blue 6B; NSB = niagara sky blue.

SUMMARY AND CONCLUSIONS

The electrophoresis experiments show that T-1824, niagara sky blue 6B, trypan blue, and niagara sky blue in serum or plasma are wholly bound by the albumin fraction when they are present in low concentrations, i.e., about 0.004 per cent or less. This no longer holds, however, when the dye concentration exceeds certain limits representing the binding capacity of the albumin. The evidence indicates that each mole of albumin can bind a maximum of 8 to 14 moles of T-1824.

Ultracentrifugation of serum albumin solutions containing 5 moles of T-1824 to 1 mole of albumin also demonstrates that the dye is bound by the protein. The dye comes down with the albumin forming a single boundary leaving the supernatant buffer solution colorless.

Addition of plasma to aqueous solutions of the dyes shifts the point of maximum absorption toward the red end of the spectrum and changes the contour of the spectral absorption curve (Gregersen and Gibson, 1937). These effects are

caused mainly by the albumin fraction (see fig. 2). There is however some difference in the effect of albumin on the absorption of the four dyes. With the two dyes, niagara sky blue and trypan blue, the optical density falls as the albumin concentration is increased to the point where its molecular concentration is $\frac{1}{2}$ that of the dye. Further increase in the albumin concentration is without much effect. With T-1824 and niagara sky blue 6B the maximal effect of the addition of albumin is reached at much lower concentrations, approximately $\frac{1}{10}$ moles of albumin per mole of dye, and the optical density then increases as the albumin concentration is raised still further (figs. 3 and 4). This ratio for the maximum number of T-1824 molecules bound by a molecule of albumin is in agreement with the value obtained from the electrophoretic studies.

The cellophane staining tests reveal differences in the affinities of the four dyes for albumin. There is a direct relationship between the rates at which these four dyes leave the circulation (Gregersen and Rawson, 1943) and their tendency to stain cellophane (see table 4). Thus although all four dyes are preferentially bound in the plasma by albumin an explanation for their different disappearance rates is found in their different affinities for albumin.

The author wishes to thank Dr. Magnus I. Gregersen for suggesting this problem and for his constant help throughout the investigation. The author is also greatly indebted to Dr. Dan Moore who made it possible to carry out the electrophoretic and ultracentrifuge studies. The expenses of these studies were, in a large part, defrayed by a grant from the Josiah Macy, Jr. Foundation.

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AN EXPERIMENTAL STUDY OF FLOW PATTERNS IN VARIOUS PERIPHERAL ARTERIES[1, 2]

R. E. SHIPLEY, D. E. GREGG AND E. F. SCHROEDER

From the Department of Medicine, Western Reserve University, Cleveland, Ohio

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During experimental studies concerning the accuracy of the thermostromuhr method for measuring blood flow (1, 2), the existence of back flow in a flow pattern was found to constitute a source of very large error in the determination of directional changes in, or quantitation of, the mean rate of flow through a vessel. It therefore became a matter of considerable interest to know in which vessels, of experimental animals, backflow normally exists and under what conditions it may appear.

Utilizing different methods, the studies of other investigators indicate, for the anesthetized dog, the normal presence (3) or absence (4, 5) of backflow in the carotid artery, its absence in the femoral (5, 6) and left coronary arteries (5), but its frequent occurrence in brachial and femoral arteries of humans (7, 8). Since experimental studies of flow patterns are not only limited but are contradictory in certain of the findings and since our own work has shown that coronary flow patterns may exhibit various degrees of backflow, an extension of the study was made to include the velocity curves of a number of peripheral arteries. These were recorded by an orifice type flow-meter (9, 10) which is adaptable to use in various body regions. Tests have demonstrated its ability to respond to frequencies of 90 to 120 D.V. per second in the blood stream, which characteristic permits adequate recording of all but the extremely rapid phasic changes in the rate of flow through the arterial vessel.

Possession of such flow patterns not only permitted selection of those arteries in which thermostromuhr flow values would be vitiated by the presence of backflow, but, of more importance, the flow curves taken together with their corresponding simultaneously recorded intravascular pressure curves afforded an opportunity for a comparative study of the flow pulses in heteronymous arteries. As the work progressed, a general system of analysis and interpretation of flow pulse patterns was attempted. Such patterns and their analysis, in so far as experimental data permit, are presented here.

PROCEDURE AND METHODS. Dogs weighing from 10 to 22 kgm. were used. The artery under study was exposed under local (procaine 2 per cent, or morphine and procaine) or general (sodium pentobarbital, sodium amytal, or ether) anesthesia; heparin, 100 units per kgm. and pontamine fast pink, 150 mgm. per kgm. were administered intravenously. An orifice-type flow meter, previously de-

[1] The expenses of this investigation were defrayed to a large extent by a grant from the Commonwealth Fund.

[2] A preliminary report of a part of this work was made before the American Physiological Society at the Chicago meeting, April 1941, and at the Boston meeting, April 1942.

signed by Gregg and Green but more recently modified by one of us (R. E. S.), was inserted between, and tied to, the cut ends of the vessel and then buried in the surrounding tissues. The existing "normal"[3] flow pattern together with the lateral blood pressure (11, 12) were recorded optically. The modifications in the flow meter were: 1. All tubular connections were made as large in diameter and as short as feasible. 2. All stop cocks were removed and the length of the flow tube was shortened to about 1.8 cm. 3. In place of the removable disc with a fixed orifice, the constriction necessary for the desired sensitivity was obtained by appropriate manipulation of an adjustable rounded stud screw, the central end of which protruded into the flow tube lumen between the lateral pressure apertures. These modifications insured not only a minimum of instrumental

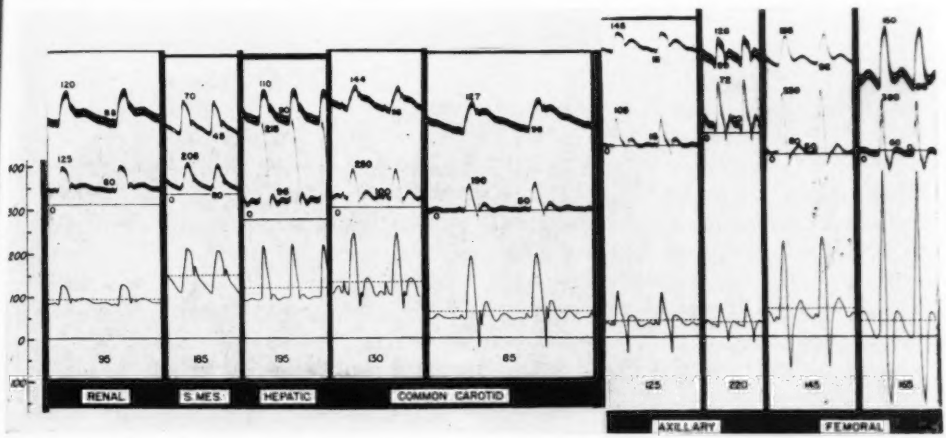

Fig. 1. Reproductions of original pressure (upper) curves and flow (middle) curves as recorded in the various arteries indicated. Lower curves are rectified reconstructions of original velocity curves. Pressure values (mm. Hg) and flow rates (cc. per min.) indicated by numbers on respective curves. Heart rate indicated by numbers at bottom of each segment. O, zero flow. Ordinate scale, flow in cubic centimeters per minute.

damping, but also facilitated the adjustment of the apparatus to any desired sensitivity during the experiment (cf. fig. 5 for details).

In 52 dogs flow patterns were obtained, usually in one and occasionally in two of the following arteries: renal, hepatic, superior mesenteric, common carotid, axillary, and femoral.

Normal patterns. Flow curves, characteristic for their respective arteries, are presented in figure 1. For clearer visualization of the flow rate/time relationship in a given curve and to permit comparisons among difference curves,

[3] In this instance, "normal" refers to those patterns recorded in dogs which have undergone only those procedures necessary for the recording of flow patterns, namely, administration of the above anesthetics and anticoagulants, and isolation of the artery and insertion of the orifice meter.

the recorded patterns have been rectified[4] and redrawn to a linear ordinate scale. These will be considered in relation to each other and to their respective pressure pulses.

Although flow curves have been obtained from a number of different dogs and recorded with different heart rates and blood pressures, flow patterns obtained in arteries supplying different body regions are subject to comparison on a common etiological basis. Examination of the large number of flow curves which have been recorded in different arteries has made possible the identification of certain distinguishing characteristics which, although easier to recognize than to describe, will be collectively examined in the following comparative and descriptive analysis.

On visual inspection, the flow patterns as recorded[5] in peripheral arteries are composed of a series of waves whose directional changes have a qualitative correspondence with gradient changes in the simultaneously recorded intravascular pressure pulses. However, the quantitative relationship of phasic rate of flow to pressure varies widely within a single cycle.

Since flow velocity varies with the differential pressure existing at the site of the flow meter, the similarity in contour of the flow and applied pressure pulses constitutes one criterion for the comparison of flow curves from different arteries and is also the basis for evaluating the influences of inherent anatomical differences among the vascular beds, the effects of which will be considered later. Certain patterns, such as the superior mesenteric and renal, retain a rather well rounded and sustained systolic portion in relative conformity to that of the pressure pulse; that of the hepatic and common carotid is less rounded while the axillary and femoral have a sharp systolic spike. The maximal pulse amplitude is seen to be small in the case of the renal and axillary patterns, somewhat larger in the superior mesenteric and hepatic, while that of the common carotid and particularly femoral patterns is quite large. Back flow is frequently present in the common carotid and consistently found in axillary and femoral patterns but has not been observed in the "normal" renal, superior mesenteric, or hepatic patterns. However, the striking feature which allows a differentiation of all the curves is the variability of the early diastolic rate of flow with respect to the presystolic rate. A comparison of this relationship with that of the corresponding early diastolic and presystolic points on the pressure curve reveals the superior mesenteric, renal, hepatic, common carotid, axillary and femoral patterns to have, in this respect, a progressively graded dissimilarity to their respective pressure curves.

[4] The original records exhibit a non-linear relationship of deflection to rate of flow in which the deflection varies exponentially with the rate of flow.

[5] The recorded patterns and rates of flow are regarded as only approximate and not exact representations of those flow changes which would have occurred had the flow meter not been inserted in the artery under study. In common with many flow meters the orifice-type meter limits flow through itself because of fluid friction within the meter unit. Hence, the amplitude of flow wave components and magnitude of mean flow changes presumably would have been slightly greater in the intact vessel than those which have been recorded and are presented here.

Flow patterns in some of the above mentioned arteries have been obtained with other methods and reported by previous investigators. Although agreeing in some respects, the patterns differ considerably from those presented here. Hewlett and Van Zwaluwenburg (7) made plethysmographic volume pulse recordings of the human forearm and arrived at flow velocity curves by differentiation of the volume curve. The presence of back flow was occasionally found normally, and invariably after nitroglycerine administration. The plethysmographic volume curves of Wright and Phelps (8) demonstrated the existence of back flow in the arterial supply of the human leg but no velocity curves were derived. Because of the difference in subjects used a common basis is not afforded for a comparison of their flow data with that presented here. Regardless of the preparation, however, it should be stressed that mean and phasic inflow values, as obtained with the plethysmograph become progressively diminished, beginning at that time when venous outflow is stopped (cuff is applied). Using the orifice meter, venous occlusion has been shown to diminish promptly the arterial inflow to the dog's leg, while at the same time augmenting markedly the normally existing back flow (13). Phasic flow records, obtained with plethysmographic method, should therefore be evaluated cautiously.

Machella (5), using a hot wire method, has presented velocity pulse curves from the femoral, carotid, and left coronary arteries of anesthetized dogs. These curves are grossly dissimilar to those shown above (and elsewhere (10, 19)) and none reveals the presence of a back flow component. Similarly, with the electromagnetic method, the carotid patterns obtained by Katz and Kolin (4) and the femoral patterns by Kolin (6) fail to exhibit back flow under normal conditions. However, Bergman (3), using the "stromborste," has published curves for the carotid of the rabbit which are quite similar to the carotid patterns (dog) presented here in figure 1.

The explanation for the pattern differences as obtained with the hot wire and electromagnetic recorders is not available. However, certain statements seem warranted. The frequency response of these instruments to changes in rate of blood flow has not been presented in the associated publications. The relatively smooth and rounded contours of the pulse waves obtained suggest that the recorded flow patterns[6] are appreciably damped representations of the actual intra-arterial velocity changes. This effect is presumably associated with the inherent characteristics of the instruments *as used*. The fact that "there is no lag in induction of an e.m.f." (Kolin, electromagnetic method (6)) does not justify the contention that "the blood velocity during each phase of the cardiac cycle is instantaneously indicated" on the graphic record. Thus, while it is granted that each of these methods may be capable of recording arterial flow

[6] It has been found that, with the orifice meter, very similar patterns can be produced rather easily by mechanical damping. If, for example, the tubes connecting the orifice tube to the optical recording unit are too long, or too small in diameter, a back flow component, known to exist in the flow pattern, may not be recorded at all or will be greatly reduced in magnitude. Similar error may follow a moderate constriction of an artery comparable to that which occurs with the application of a snugly fitting thermostromuhr unit, and which may occur when the electromagnetic unit is applied.

patterns, experimental tests of the response of the *entire* working instrument is known rapid fluctuations in flow velocity are needed before the validity of the recorded patterns can be established.

ANALYSIS AND INTERPRETATION. The foregoing peripheral patterns (fig. 1) reveal the existence of wide variations in volume, timing, direction and rate of flow. With the exception of the work of Hewlett and Van Zwaluwenburg, flow patterns have been considered primarily from a descriptive standpoint. While the recorded data for this and a companion paper (18) were being assembled and studied, a semi-quantitative method of analysis was evolved which was found to facilitate the recognition and identification of flow patterns of various peripheral arteries.

It became apparent early in the study that, for a given bed, the character of the venous outflow pattern was an important consideration in the analysis of the arterial inflow pattern. Venous flow patterns were recorded in the femoral, renal, and *high* in the external jugular veins under control conditions and following the injection of vasodilator drugs. The phasic variations[7] in the rate of venous flow were never found to be greater than 5 per cent of the mean flow value. Venous phasic flow studies found in the literature have been largely confined to the head circuit. Direct observations (14) have shown that flow pulsations in the capillaries and small veins of the rabbit's ear are almost nil.

These and our findings do not necessarily conflict with the observations of Burton-Opitz (15) and Holzlöhner (16) who found that the external jugular flow pattern is markedly pulsatile. Flow patterns very similar to those of Holzlöhner have been obtained with the orifice meter when the instrument was inserted into the lower portion of the vein (in the region of the supraclavicular fossa). Because of the proximity to the heart and the marked phasic changes in the recorded venous pressure, we concur in the belief (15, 16) that the pulsatile flow waves obtained in this portion of the vein are (central) cardiac in origin rather than (peripheral) waves transmitted through the capillaries. Our conception of the probable determinants of flow *pattern* and the method by which they were identified are illustrated in the following stepwise analysis of a sample flow pattern.

In figure 2 are shown reconstructions of a femoral arterial flow pattern and pressure curve, recorded as previously described for similar femoral patterns shown in figure 1. Under conditions of reasonable constancy the mean rate of arterial inflow during a given cycle is accepted as representing the existing mean rate of blood flow through the corresponding bed. This is indicated by the line *MM*. The areas representing volume flow, which lie above (*B*, *E*) and below (*A*, *C*, *D*, *F*) the mean flow line are equal by virtue of the mean position occupied by the mean flow line, *MM*. Since, aside from the comparatively

[7] Although these are conceivably remnants of the original flow pulse which has traversed the capillary bed, they are possibly artifacts resulting from generalized arterial impact phenomena, or more probably flow responses to cyclic fluctuations in the central venous head of pressure. In support of the last mechanism it was observed that simultaneously recorded venous pressure and mean flow waves underwent reciprocal directional changes.

slow respiratory fluctuations (17), the femoral venous flow pattern exhibits only very small cyclic variations and is essentially a smooth, "linear" flow curve, it corresponds very closely to the straight line MM, which is used as its graphic representation in this analysis. It therefore follows that from the point of recording arterial inflow to that of recording venous outflow (line MM), the flow pattern has been smoothed out, presumably by viscous resistance to flow and volume-elastic moderation. In the following portion of the analysis, the rela-

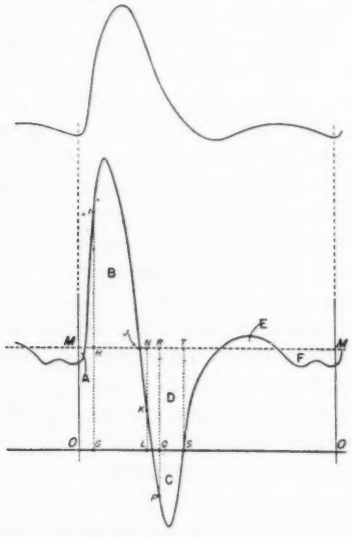

Fig. 2

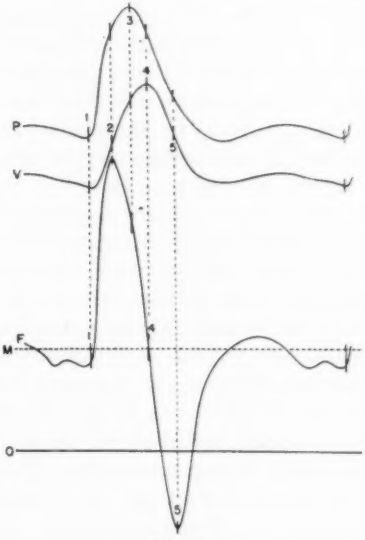

Fig. 3

Fig. 2. Upper curve—Reproduction of femoral arterial pressure pulse. Lower curve—Reconstructed flow pattern, rectified from original optical recording. Interrupted line MM, mean flow level. Line OO, zero flow level. Areas B, E, volume-elastic increments. Areas A, C, D and F, volume-elastic decrements. C, area of back flow. For other notations, see text.

Fig. 3. Same flow (F) and pressure (P) curves as shown in figure 2 with derived intravascular volume curve, V. M and O, same as in figure 2. Synchronous vertical intercepts on each curve, reading consecutively, are: 1, onset of flow and pressure rise; 2, maximal rates of forward flow and volume increase; 3, peak of applied pressure; 4, point of maximal intravascular volume; 5, maximal rates of back flow and volume decrease. Further explanation in text.

tionship of the pulsatile arterial inflow curve to its corresponding essentially "linear" venous outflow curve reveals the basic mechanism responsible for the recorded phasic variations in rate of inflow (flow pattern).

Since an arterial vascular tree is functionally an expansible chamber as well as a tubular conducting system, application of a pulsatile pressure head will give rise to a pulsatile change in the contained volume of that arterial tree. The significance of the volume changes may be illustrated in figure 2. The area

enclosed by the mean flow line (*MM*), zero flow line (*OO*), and the two intercepts marking the beginning and end of one cycle (*MMOO*) represents the volume of blood passing through the bed during a given cycle. Since the venous outflow pattern can be regarded as essentially a straight line (*MM*), the areas *B* and *E* above the mean flow line represent a certain volume of blood accommodated in the arterial tree *in excess* of that leaving the bed during the same time interval. Furthermore, the areas (*A*, *C*, *D*, *F*) below the mean flow line represent an *equal* volume of blood which the vascular tree does not accept during the same cycle as the necessary accompaniment to the accommodated excess. In fact, during the early systolic portion of the cycle the vascular tree has accommodated a volume greater than that which can run off peripherally during the late systolic and early diastolic portion, and a back flow of blood is recorded. In the flow curve illustrated, the amount of excess which cannot immediately participate in the peripheral run off is indicated as a definite back flow volume (cf. area *C*). However, as will be shown in a subsequent communication (18), as the mean flow through a bed becomes sufficiently large the systolic excess is compensated by a period of decreased forward flow rather than by a period of back flow. On the other hand, if the mean arterial inflow becomes smaller the volume of back flow per cycle is increased and the forward flow component is decreased. Under conditions in which the mean rate of flow is quite small the flow pattern may indicate the forward flow increment to be almost entirely canceled out by the back flow decrement. Thus, the pulsatile flow waves (fig. 2—*A*, *B*, *C*, *D*, *E*, *F*) which necessarily accompany the pulsatile volume changes peripheral to the orifice meter can be regarded as the volume-elastic (V-E) flow components. These oscillatory components of the inflow pattern therefore represent the changes in the rate of flow to the distensible arterial reservoir through which are mediated the corresponding phasic changes in intravascular volume (volume-elastic volume changes within the bed).

Using the same arterial inflow pattern (fig. 2) the analysis can be extended to the consideration of single points on the inflow curve in relation to simultaneous points on the venous outflow curve. An algebraic summation of the two reveals the contribution made to the inflow curve by the "volume-elastic flux" of blood within the arterial bed. Considering first, point *I* in figure 2 on the ascending systolic flow limb, the distance *GI* represents the rate of inflow recorded at the flow meter; *GH* represents the mean rate of flow through the bed, and *HI* the rate of V-E "flow of accommodation" within the bed. At point *J*, the inflow, mean rate of flow, and outflow are the same, indicating no V-E volume change within the bed. At point *K* the distance *KL* indicates a small rate of inflow. Since *NL* shows the outflow rate to be as large as *HG* (at point *I*) it must be assumed that *NK* represents the rate at which the arterial bed decreases in volume (recoil) to maintain the same rate of outflow. At point *P*, the rate of V-E recoil, *RP* is so great that a backflow of blood progressing at the rate of *PQ* occurs in spite of the constant outflow, *QR*. At point *S*, the rate of recoil, *TS*, is equal to the outflow rate *ST* and therefore the rate of inflow at the meter is equal to zero. Such an analysis should hold for any peripheral

arterial bed where the arterial inflow pattern is known and the venous outflow is essentially nonpulsatile.

It may therefore be stated that: 1. The rate of venous outflow from a peripheral bed, under reasonably constant conditions, is identical in magnitude to the mean rate of arterial flow into the corresponding bed, which value, in turn, determines the *placement* of the flow pattern above the zero flow line. 2. The algebraic difference between the rate of arterial inflow and the rate of venous outflow expresses the rate and direction of "V-E flow," of which the flow pattern *contour* is a continuous graphic representation. The "V-E flow," although recorded as an axial flow, is actually a radial displacement of blood within the vessels. The direction of the radial displacement (centripetal or centrifugal) will depend upon the pressure gradient at the moment. The pressure gradient is, in turn, determined by the character of the applied pressure pulse (central to the flow meter) and the V-E properties of the arterial tree and adjacent extravascular tissues (peripheral to the meter). 3. Whenever the rate of retrograde "V-E flow" (centripetal intravascular recoil) *exceeds* the rate of peripheral outflow, the arterial flow pattern will reveal a backflow component. The magnitude of the backflow will depend upon the duration and magnitude of such an *excess*.

The phasic fluctuations in intravascular volume versus the accompanying phasic fluctuations in applied pressure head constitutes a special type of V-E relationship. Unlike a "static" V-E (SV-E) relationship which exists only under static conditions of equilibrium, the relationship here of a changing volume to a changing pressure exhibits several important differences. The latter may be attributed to the influences of inertia and viscosity which appear only in the "dynamic" state of pressure-flow-volume change, and whose effects will be considered later.

The determination of a V-E curve under static conditions (SV-E curve) which will have reliable significance under normal experimental (dynamic) conditions *in vivo* does not seem possible at present. However, it appears justifiable to evaluate changes in the flow pattern in terms of the probable and logically anticipated changes undergone by the theoretically existent SV-E curve.

Within limits it would be expected that the net effects of vasoconstriction and dilatation will be that of decreasing and increasing respectively the intravascular volume change which will result from a given change in distending pressure. The recorded changes in flow patterns presented elsewhere (18) contribute support to this belief. In the case of vasoconstriction the effect on the SV-E curve will be that of displacing it away from the volume axis, while dilatation, increasing the sensitivity of volume response, shifts the curve toward the volume axis.

As outlined previously, the cyclic volume changes within the arterial bed are manifested in the inflow pattern as the V-E components. It therefore will be expected that vasoconstriction will have the effect of diminishing the magnitude of the V-E flow components (see areas *A*, *B*, *C*, *D*, *E* and *F*, fig. 2) thereby indicating smaller cyclic volume changes within the bed. Similarly, vasodilatation will permit a greater cyclic volume change within the bed and there-

fore V-E flow components of greater magnitude in the flow pattern. The effects of vasomotor drugs upon the flow pattern have been recorded and are presented in a companion paper.

In continuation of the flow pattern analysis, it becomes evident that "static" V-E relationships alone are inadequate for an evaluation of the constantly changing or "dynamic" pressure-flow-volume relationships existing within the period of a single flow cycle. The dynamic factors of inertia and viscosity introduce a considerable variation in the magnitude and timing of the intravascular volume response to applied pressure change; as the result of inertia the volume change may lag behind or possibly overshoot its corresponding change in applied pressure while viscosity imposes a considerable damping and retardation of the volume change.

The magnitude of the combined effects of inertia and viscosity can be evaluated from the same flow and pressure curves reproduced in figure 3. By graphic integration of the velocity curve, *F*, the corresponding volume pulse curve *V* has been constructed.[8] The fact that the intravascular volume change is not synchronous with the applied pressure change is revealed by the temporal disagreement between comparable segments of the pressure and volume curves. The relationship of pressure to volume can be better visualized when synchronous points on the two curves are plotted against each other, cf. figure 4. In this form the dynamic V-E relationship appears as a continuous circuitous curve. Parallel to the long axis of the curve and passing through its apices is line *S*, which has been inserted to represent a conceivable and not improbable general position of the "simultaneously existing static V-E curve."[9]

In relation to the *SV-E* curve, the *DV-E* curve is displaced, first, away from the volume axis during the major pressure rise, and then toward the volume axis

[8] No means are available for determining, in a given bed, the contained volume of blood between the flow meter and the point wherein flow pulsations are essentially nil. While curve *P* indicates the applied pressure changes which exist above a known diastolic level, curve *V* will show the intravascular volume changes which are superimposed upon an unknown total intravascular volume. Therefore, a graph of applied pressure versus intravascular volume increment (fig. 4) will represent the "dynamic" relationship of applied pressure (not distending pressure) to volume change without reference to absolute position along the volume axis. It is realized that the applied pressure, which is recorded at the meter, bears no constant relationship to the mean distending pressure throughout the arterial tree and further ignores the presence of those changes which may occur in intracapillary pressure.

[9] The "static V-E curve" referred to here could be experimentally determined only if it were possible to remove suddenly from the arterial bed all dynamic and physiological influences, i.e., flow through the bed with its viscosity and inertia effects, nervous and metabolic influences upon the size and distensibility of the vessels, etc. In this hypothetical state a single static V-E curve could be determined at leisure which would represent the pressure-volume relationship for the same bed, with the same vasomotor and elastic state of the vessels but without the two dynamic influences of viscosity and inertia. The basic, although theoretical, existence of this "static V-E relationship" in a dynamic system is a concept essential to this analysis. However, the precise position and exact slope of its graphic representation (curve *S*, fig. 4) need not be known for the illustrative and comparative purposes for which it is used here.

as the pressure is falling. Both displacements indicate that the volume change lags behind the pressure change. While it is impossible to identify and separate the inertia and viscosity effects, the latter appear to exert the greater dynamic influence and will be considered in more detail.

As applied pressure and flow velocity rise at the beginning of the cycle (see figs. 3 and 4) the increase of intravascular volume lags behind the rise in pressure. Similarly, during the fall of the major pressure wave the rate at which the volume of the bed decreases is likewise retarded. Since the pulsatile volume

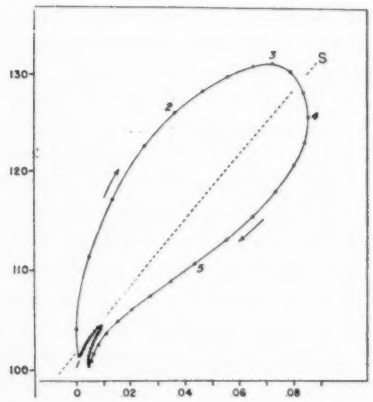

Fig. 4

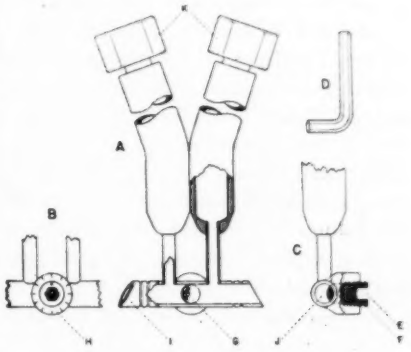

Fig. 5

Fig. 4. Graph showing a continuous plot ("dynamic" V-E curve) of applied pressure versus intravascular volume increment derived from flow curve *F*, figure 3. Ordinate, applied intra-arterial pressure in millimeters Hg. Abscissa, intravascular volume increment in cubic centimeters. Dots on curve demarcate uniform time intervals (1/50 cycle length). Numbered dots indicate time relations of correspondingly numbered intercepts in figure 3. Arrows indicate direction of progression with time. Interrupted line *S*, presumptive trend of "static" V-E curve.

Fig. 5. Semi-diagrammatic sketch of modified orifice type flow meter with readily adjustable orifice. *A*—back, *B*—front, and *C*—side views of meter with cut-away sections showing relations of internal parts. *D*, key wrench for adjusting size of orifice. *E*, key socket. *F*, threaded stud screw with hemispherical end protruding into lumen of tube. (See end view, *G*.) *H*, circular scale for orifice settings. *I*, end, cannula-shaped. *J*, lumen of meter tube. *K*, female connections for attachment to optical differential pressure recorder.

changes are mediated through pulsatile changes in rate of flow to the bed, it is reasonable to believe that the former will be diminished or damped in proportion to the extent to which frictional resistance diminishes or damps the flow of blood to, and exchange of blood within, the bed. The net effect upon the applied pressure-intravascular volume relationship (fig. 4) can be summarized as an inability of the volume change to "follow" the rapid applied pressure change, due to the high viscous damping within the fluid system.

In summarizing to this point the foregoing analysis as applied to the original

flow pattern (fig. 2) or to any other peripheral arterial flow pattern, the following may be said: the flow curve is a record of the direction and velocity with which blood flows by a point in a peripheral artery supplying a given bed. Since the peripheral outflow from the same bed is essentially nonpulsatile, variations in the rate of inflow must reflect the capacity of the arterial tree to distend and recoil during the cyclic rise and fall of the applied pressure pulse. Basically, the theoretical volume which can be accommodated for a given rise in pressure (SV-E relationship) is determined by the existing vasomotor state and elastic properties of the arterial vascular tree. To deliver that theoretical volume throughout the tree in a dynamic system it would be necessary that the inflow acquire a velocity of sufficient magnitude so that the volume to be accommodated is delivered within the period of time during which pressure is rising. In the reverse situation, as the applied pressure is falling, the amount by which the intravascular volume diminished should be compensated by an appropriate and simultaneous decrease in the rate of inflow. However, such theoretically ideal circumstances do not prevail in a dynamic system since fluid friction (viscosity) diminishes greatly the velocity with which blood flow responds to pulsatile changes in applied pressure. Consequently, the intravascular volume *response* lags behind and undoubtedly fails to reach its theoretical value based upon the existing applied pressure. This damping effect is reflected in the inflow pattern as a diminution in the magnitude of deviation from the mean rate of flow and in the rapidity with which flow responds to change in applied perfusing pressure.

Extending further the analysis of flow pattern, a consideration of conditions in the experimental animal reveals that several factors, collectively and interdependently, may determine the *extent* of viscous damping which will exist *at any given moment* in the cycle. Divided into two classes, they are those which affect 1, the velocity of flow, and 2, the intravascular bore (as it determines internal surface area/volume relationship). In class I are *a*, the magnitude of volume flow through the bed (position of pattern above zero flow level), and *b*, rate of change of applied pressure. In class II are *a*, active or passive constriction, and *b*, active or passive dilatation of the arterial vessels. The magnitude of viscous damping will vary in the same direction as do the changes in 1*a*, 1*b*, and 2*a*, and in the reverse direction from those in 2*b*. However, the magnitude and direction of the effects of a single variable are frequently unrecognizable and may be complicated by associated changes in other variables. For instance, decreasing intravascular bore by active vasoconstriction (2*a*) will augment viscous damping by increasing the internal surface area/volume relationship, but under certain circumstances the effect may be partly or completely nullified, or even over-compensated by an accompanying decrease in mean flow (1*a*) and/or an increase in the pressure pulse. The latter will induce a certain degree of passive mechanical dilatation (2*b*) at the higher pressure values. It will elicit not only an absolute increase in intravascular volume *exchange* by virtue of the greater pressure extremes, but also a relative decrease because of the accompanying increase in the rate of change of applied pressure (1*b*). The same considerations may be applied to the oppositely directed changes associated with active dilatation of the arterial vessels.

The interrelationship of the above factors is of such complexity that the effects of a given variable can be evaluated only with respect to the net effect induced by it and other dynamically associated factors (18).

Unfortunately the above analytical approach does not encompass all of the factors which comprise the determinants of coronary flow patterns. The latter are influenced by the effects of cyclic changes in intravascular volume associated with compression and relaxation of extrinsic origin (extravascular support). In addition coronary venous outflow is markedly pulsatile. Consideration of coronary flow patterns will be made in a future communication.

SUMMARY

Flow patterns (and simultaneous intra-arterial pressure curves) have been optically recorded with an improved orifice-type flow meter in the renal, hepatic, superior mesenteric, femoral, axillary, and common carotid arteries of dogs to which has been administered only anesthetic and anticoagulant.

A flow pattern may be characteristically distinctive of a given artery and its bed, but flow patterns in heteronymous arteries are found to exhibit wide variations in magnitude, timing, direction, and rate of flow and in similarity of contour to their respective pressure pulses. Back flow components have been consistently found to exist in the femoral and axillary arterial flow patterns, frequently found in the common carotid patterns, while the renal, hepatic, and superior mesenteric have exhibited only forward flow.

A study has been made revealing the probable determinants of, and their interrelated influences upon, the phasic rate of inflow to a bed (flow pattern) under the above and other physiological conditions.

Although the above analysis does not lend itself to a *quantitative* evaluation of the static and dynamic factors which initiate and moderate the phasic rate of inflow to a bed, it constitutes a basis for a *qualitative* evaluation of differences among, and changes in, flow patterns recorded in the same or different arteries under various physiological conditions.

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A STUDY OF FLOW AND PATTERN RESPONSES IN PERIPHERAL ARTERIES TO THE INJECTION OF VASOMOTOR DRUGS[1, 2]

W. H. PRITCHARD, D. E. GREGG, R. E. SHIPLEY AND A. S. WEISBERGER

From the Department of Medicine, Western Reserve University, Cleveland, Ohio

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In a previous communication (1) there were presented a method of analysis and interpretations of flow patterns recorded in various peripheral arteries of the anesthetized dog. In association with that study flow patterns have been recorded under a great variety of conditions. Some of the more interesting pattern changes have been observed to follow the injection of vasomotor drugs. The phasic flow curves and their interpretations presented here will reveal, in part, those dynamic changes which occur locally as well as those occurring in the general circulatory system.

The preparation used here was similar to that previously described (1) and, in brief, consisted of an anesthetized dog to which antigoagulant had been administered. Phasic arterial pressures and flow rates were recorded simultaneously and continuously by the optical methods of Gregg and co-workers (1, 2, 3, 4, 5).

Transformations in flow pattern and changes in mean rate of blood flow were induced in the renal, superior mesenteric, hepatic, common carotid, axillary and femoral arteries by the injection of vasodilator and vasoconstrictor drugs. The drugs were injected either intravenously or intra-arterially and continuous records were taken. By the latter route local and general systemic effects could be temporarily separated so that the immediate local effects upon flow and pattern could be recorded before the delayed and more general systemic effects appeared. Following the intravenous injection the combined effects of the local and general systemic changes on the magnitude of flow and pattern were observed to appear more nearly simultaneously.

In the interpretation of changes in the flow curves to be presented here the significant considerations are those which have been previously set forth (1): 1, the mean height of the flow pattern above the zero flow level which indicates the magnitude of mean rate of flow through the bed, and 2, the volume of pulsatile deviation from the mean flow line which reflects the dynamic volume-elastic properties of the arterial tree.

In addition, vasomotor changes within a bed can be recognized under certain conditions by correlating, as a simple ratio, the mean flow value with the corresponding mean applied pressure value. However, the application of such an index is limited and should be made with caution. Such an index does not take

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into account viscosity effects which, in a dynamic system, may give rise to considerable variation in the relationship of mean pressure to mean flow, for which appropriate *in vivo* correction curves are not obtainable. Nevertheless, it is believed that the vasomotor state of a bed may be regarded as having changed when mean flow and mean blood pressure undergo considerable change in opposite directions.

As recorded in the inflow pattern, the pulsatile deviation from the mean rate of flow (dynamic volume-elastic flow components) varies directly with the pulsatile differential pressure at the site of the meter. However, the magnitude of the volume flow represented by these waves is limited by the extent to which fluid friction (viscous damping) retards the rate of intravascular volume exchange (dynamic volume-elastic "flow") of blood. The interpretation of changes in the flow patterns will deal with the interrelationship of the above factors whose respective determinants and mode of operation have been presented in a previous analytical study (1).

A condensed description and interpretation of typical transformations in flow pattern found to occur in various arterial beds following the injection of drugs is presented below. For better evaluation of flow pattern changes the curves have been rectified to a linear scale and the mean flow level indicated. In some instances reproductions of the original flow curves are also included.

Intra-Arterial Drugs. Renal artery—nitroglycerine (fig. 1A). Mean flow first increases and then decreases as the local and more general systemic vascular responses appear, respectively. The dynamic volume-elastic (DV-E) component, while relatively unchanged at 22 seconds, soon becomes larger and later quite small. The appearance of back flow has never been observed in the renal artery following the injection of vasodilator drugs.

The explanation for these flow and pattern changes is reasonably clear. The renal vascular bed obviously dilates initially (since blood pressure falls but mean flow increases). Dilatation possibly continues throughout the period of fall of blood pressure but is difficult to establish with certainty since both blood pressure and blood flow change in the same direction. Since at the time of maximal mean flow (22 sec.) the magnitude of the DV-E flow components is not appreciably altered, it is suggested that the accompanying increase in rate of flow has imposed a relatively greater viscous retardation of DV-E flow (intravascular volume exchange). The effect of a diminished rate of flow in decreasing viscous damping is suggested in the record taken 54 seconds after injection when the DV-E components are much larger than the control in spite of a smaller pressure pulse.

Renal artery—suprarenin (fig. 1B). Mean flow is promptly reduced, almost to zero. The DV-E components become very small in spite of an increased pulse pressure and the flow pattern is grossly changed and transformed into a number of small, sharply angular oscillations, some of which descend below the zero flow line, thereby constituting periods of back flow. The direction of each flow fluctuation is in phase with a corresponding rapid change in pressure gradient. (Note transitional stages in 1–2 sec. record.)

It is evident that local vasoconstriction is marked. A reduction in DV-E

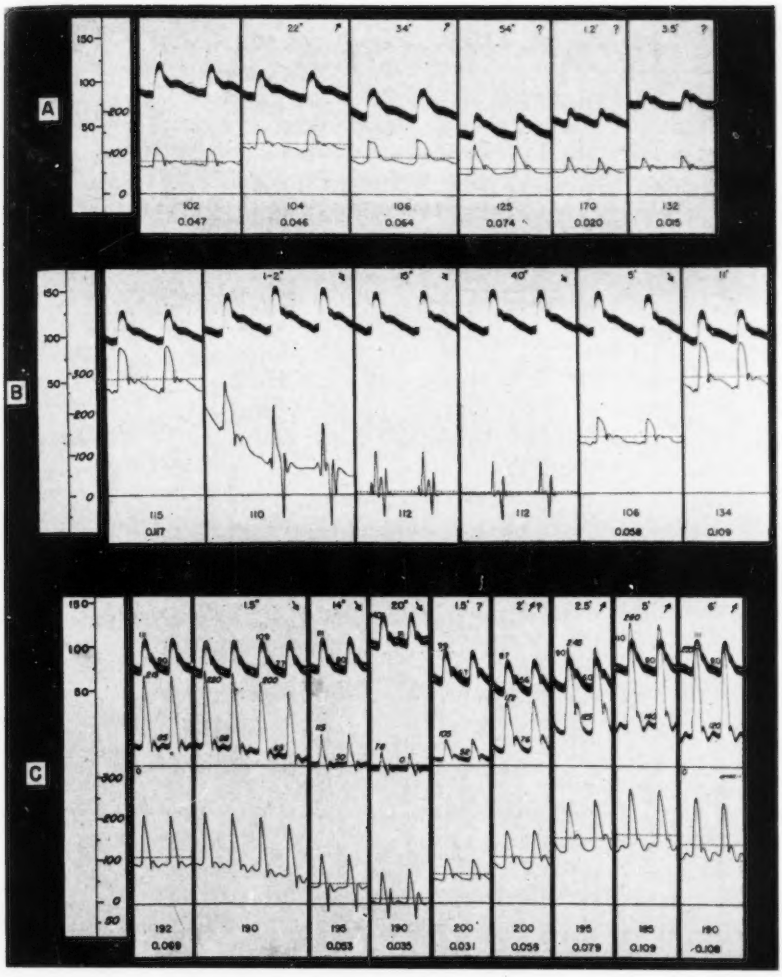

Fig. 1 A. Reproductions of a series of original pressure (upper) curves and rectified reconstructions of original velocity flow (lower) curves recorded in the renal artery following the intra-arterial injection of nitroglycerine (1.2 mgm.). Dog weight 15.4 kgm. Ordinate scales—pressure in millimeters of mercury on left. Flow in cubic centimeters per minute on right. Continuous horizontal line indicates zero flow level. Interrupted line indicates mean flow level for each record segment. Numbers on each record segment, reading consecutively from top to bottom are: time elapsed following drug injection, heart rate, and volume-elastic flow component (increment or decrement) in cubic centimeters per cycle. Change in vasomotor state of bed with reference to control state is indicated in right upper corner of record segment by: ↗-dilatation, ↘-constriction, ? - not known.

Fig. 1 B. Renal arterial pressure (original) and flow (rectified) curves showing serial effects of intra-arterial injection of suprarenin (0.05 mgm.). Dog weight 15.4 kgm. Order of curves, notations, and ordinate scales same as figure 1 A.

Fig. 1 C. Reproductions of original pressure (upper) and flow (middle) curves with rectified flow curve reconstructions (lower) recorded in the hepatic artery following the intra-arterial injection of suprarenin (0.10 mgm.). Dog weight 12.7 kgm. Numbers on pressure curves indicate systolic and diastolic pressure values in millimeters of mercury. Slanting numerals on original flow curves indicate late diastolic and maximal systolic rates of flow in cubic centimeters per minute. Other notations and ordinate scales same as figure 1 A.

components exists in spite of an increased pulse pressure and lowered mean flow, and occurs, presumably, as a result of diminished elastic capacity of the constricted vessels and the augmented viscous retardation imposed by the associated reduction in bore.

Hepatic artery—suprarenin (fig. 1C). The changes in flow pattern, the appearance of back flow, and the reduction in DV-E component during vasoconstriction are quite similar to those found in the renal artery and are to be ascribed to the same influences. On the basis of the criteria set forth above, the vasomotor change in the bed at 1.5 and 2 minutes is that of dilatation with reference to the 20 second record but its state cannot be determined with respect to the control state, since both blood pressure and mean flow have changed in the same direction. Later, during the recovery phase, marked vasodilatation appears and is accompanied by an increased DV-E component. The most important determinant of the augmented DV-E fraction is presumably the ability of the dilated vascular bed to undergo greater pulsatile volume changes. The secondary dilatation may also appear in the renal artery but does not invariably occur in either artery.

Common carotid artery—nitroglycerine (fig. 2A). Mean flow first increases and then drops (as blood pressure falls) toward the control value. The pattern most nearly resembles the contour of its pressure pulse at the maximal rate of flow, this resemblance diminishing as blood pressure and flow decline. The DV-E component, relatively unchanged as mean flow increases, becomes very large as blood pressure falls and a small back flow appears in early diastole.

Local dilatation is immediate and persists throughout the 2 minutes of records. The DV-E changes have a similar origin to that described for the renal artery following injection of the same drug. The appearance of back flow is obviously associated with the increased amplitude of the DV-E component occurring in conjunction with a lowering of the mean flow level.

Similar but more prolonged effects are obtained following the injection of aminophylline, with the exception of the fact that the fall in blood pressure and mean rate of flow is not as great and no back flow appears (records not shown).

Common carotid artery—suprarenin (fig. 2B). Mean flow is progressively reduced and remains low despite the subsequent large elevation of blood pressure. The flow pattern contour undergoes only minor changes initially but, as blood pressure and pulse pressure increase, the flow pulse amplitude and DV-E component increase greatly and several back flow components of considerable, though varying, amplitude and magnitude appear during diastole. The appearance of back flow with diminished mean rate of flow has been previously observed by Katz and Kolin (6).

Marked vasoconstriction is obviously present. The increase in the DV-E component, in contrast to that following the injection of the same drug in the renal and hepatic arteries, is probably related to the dominant influences of the markedly increased pulse pressure and decreased mean flow over the combined damping effects of reduced vascular bore and increased rate of DV-E flow.

Comparable results are obtained with pitressin except that, since the pressure

pulse is only mildly elevated, the flow pulse amplitude and DV-E fraction are only moderately increased (records not shown).

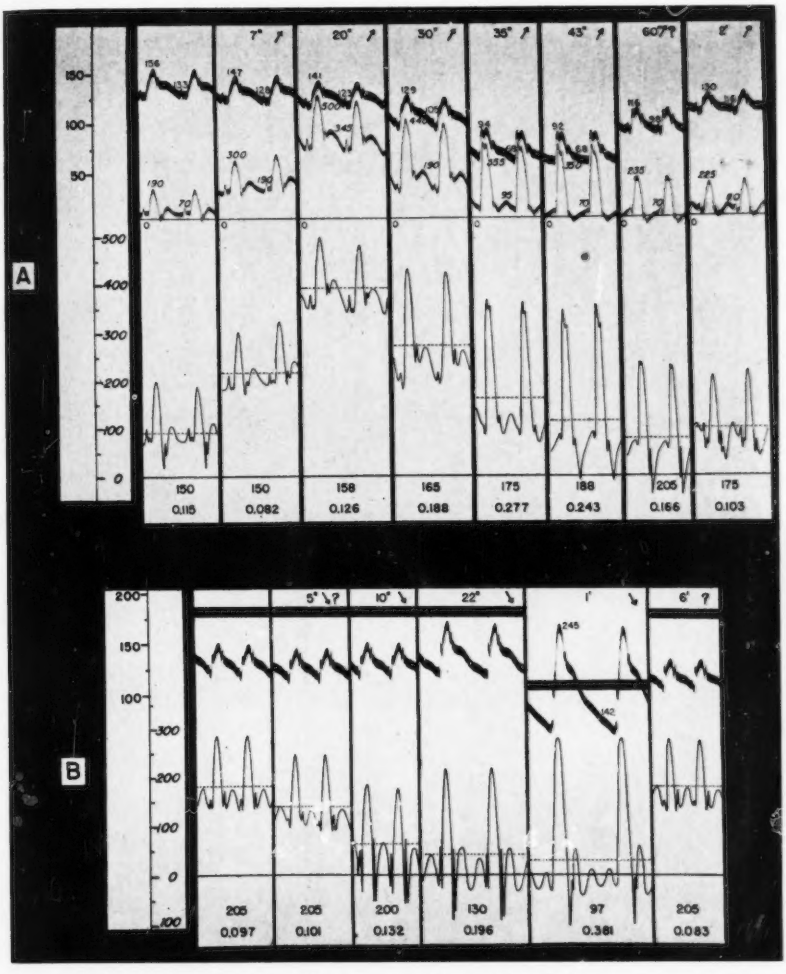

Fig. 2 A. Original pressure and flow curves with rectified flow curve reconstructions recorded in common carotid artery following intra-arterial injection of nitroglycerine (0.3 mgm.). Dog weight 12.6 kgm. Order of curves, notations, and ordinate scales same as figure 1 C.

Fig. 2 B. Common carotid arterial pressure (original) and flow (rectified) curves recorded following intra-arterial injection of suprarenin (0.1 mgm.). Dog weight 13.4 kgm. Order of curves, notations, and ordinate scales same as figure 1 A.

Femoral artery —nitroglycerine (fig. 3A). As with responses to vasodilator drugs in other arteries, mean flow at first increases and then decreases. Both

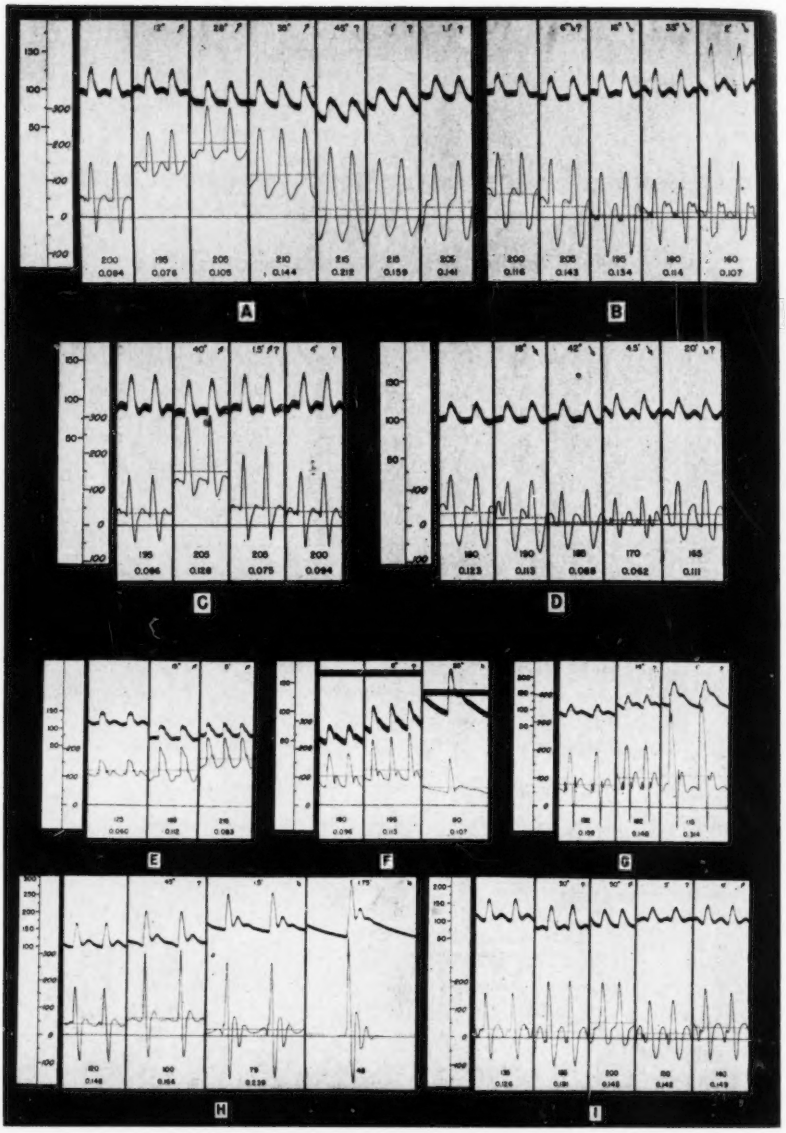

Fig. 3 A through D. Femoral arterial pressure (original) and flow (rectified) curves recorded following the intra-arterial injections of vasodilator and constrictor drugs. Part A—nitroglycerine (0.3 mgm.). Dog weight 11.2 kgm. Part B—suprarenin (0.1 mgm.). Dog weight 13.4 kgm. Part C—aminophylline (24 mgm.). Dog weight 13.5 kgm. Part D —pitressin (12 units). Dog weight 13.4 kgm. Order of curves, notations and ordinate scales same as figure 1 A.

Fig. 3 E through I. Reproductions of pressure curves (original) and flow patterns (rectified) recorded in different arteries following the intravenous injections (via external jugular) of vasodilator and vasoconstrictor drugs. Part E—renal artery, theamin (250 mgm.). Dog weight 14.0 kgm. Part F—hepatic artery, suprarenin (0.2 mgm.). Dog weight 12.7 kgm. Part G—common carotid artery, suprarenin (0.06 mgm.). Dog weight 10.4 kgm. Part H—femoral artery, suprarenin (0.07 mgm.). Dog weight 11.4 kgm. Part I—femoral artery, nitroglycerine (0.3 mgm.). Dog weight 11.4 kgm.

systolic and diastolic portions of the flow pattern become full, more rounded, and the "normally" existent back flow disappears with the elevation of mean flow. As mean flow (and blood pressure) drop the DV-E component increases markedly and back flow of considerable magnitude reappears, occupying most of diastole. The changes in pattern and the mechanisms responsible for them are similar to those described for the effects of nitroglycerine in the renal and common carotid arteries.

Injection of aminophylline (fig. 3C) produces changes which are smaller but quite similar to those occurring initially after injection of nitroglycerine, i.e., mean flow increases, obvious dilatation occurs, and the back flow disappears, but the flow pattern and the DV-E fraction are not greatly altered.

Femoral artery—suprarenin (fig. 3B). The mean flow rapidly reaches a low level and remains low despite the subsequent marked augmentation in blood pressure. The flow pattern is only moderately altered in contour but the back flow is of greater amplitude and volume. The DV-E fraction at first increases mildly, but later, at 2 minutes, returns to the control value despite a large pulse pressure. This response contrasts with that observed in the common carotid but compares with the effects observed in the hepatic and renal arteries.

It is evident that relatively marked vasoconstriction appears and persists. Again the relatively small change in the DV-E component is regarded as evidence of a close balance among the effects of increased pulse pressure, decreased mean flow, altered distensibility of the vessels and a greater viscous damping because of their constricted state.

Similar changes in pattern follow the injection of pitressin (fig. 3D) except that a considerable reduction in the DV-E component occurs. This is undoubtedly associated with the failure of the pulse pressure to become augmented sufficiently to offset the damping effects of the same factors as mentioned above.

Correlation of Effects of Drugs Injected Intra-arterially. As anticipated, so-called vasoconstrictor and vasodilator drugs given intra-arterially have caused, in the local bed, vasoconstriction and vasodilatation respectively, i.e., the mean flow has decreased at a higher blood pressure, or increased at a lower blood pressure. However, in some of the above records the systemic effects of the drug have so affected the applied perfusing pressure that these criteria alone are not applicable to reveal changes in the vasomotor state of the bed. Such a shortcoming is particularly evident when interpreting changes following the intravenous injection of drugs.

Certain generalizations can be made regarding the transformations in flow patterns recorded in various peripheral arteries. The existence of a V-E flow component is independent of the mean rate of flow through the bed. Its magnitude may be influenced by variations in the mean rate of flow, but as Hewlett and Van Zwaluwenburg (7) have pointed out, directional changes in either may occur independently of the other. In the early stages following the intra-arterial injection of vasodilator drugs, before the systemic effects appear, the arterial pattern acquires a contour which resembles more nearly that of its corresponding pressure pulse. For a given artery, the similarity of pressure and flow patterns is frequently observed to be greatest when the mean rate of flow is high and

viscous damping of the volume-elastic exchange of blood is thereby presumably increased. Conversely, the similarity is usually least when mean flow is low and the volume-elastic exchange is less damped.

In comparing the drug effects observed in different arteries it must be kept in mind that all beds do not possess the same sensitivity of vasomotor response. In addition, the total volume of the vascular bed will vary in different organs and extremities as will the distribution of that volume among vessels, such as the arterioles, which are relatively more responsive to vasomotor drugs and those vessels, such as the large arteries, which are less responsive. A comparison of the flow patterns obtained in the renal and common carotid arteries following the intra-arterial injection of suprarenin (fig. 1B, fig. 2B) illustrates the inherently different responses in the two beds. In other experiments, renal inflow immediately and rapidly fell to a very low level following a very small injection (of the order of 0.0001 mgm.), while a much larger dose in the common carotid (approximately 0.1 mgm.) was much less prompt and effective in its local constrictive action (records not shown). The marked decrease in the DV-E component observed in the renal, in contrast to the large increase in the common carotid, is undoubtedly associated with the smaller accommodative capacity for volume-elastic exchange in the former. The short length of the renal artery and small total arterial bed volume with a relatively large proportion of arterioles in the kidney constitute a comparatively small reservoir which, in addition, is capable of inducing considerable viscous damping (of flow fluctuations). On the other hand, the common carotid possesses a relatively long system of large arterial branches, a large vascular bed and, presumably, a smaller proportionate volume of arterioles. Thus the effects of arteriolar constriction upon the DV-E component may be easily lost in, and overpowered by, the influence of even a small systemic augmentation of pulse pressure upon the more elastic and accommodative large arterial vessels which impose only a relatively small viscous limitation upon DV-E flow fluctuations and undergo comparatively smaller degrees of constriction. The pattern responses observed in the other arteries appear to fall between the above extremes and vary in relation to the physiological sensitivity and anatomical distribution of the vascular components.

Similarly, the effects of dilator drugs upon the arterial inflow patterns will depend upon the preëxisting vasomotor state of the bed and the same physiological and anatomical factors as those mentioned above.

It is interesting to note that the injection of *either* constrictors or dilators may be associated with the appearance or augmentation of back flow in many arteries. Its existence in any artery may be ascribed to the presence of a single set of conditions, irrespective of the artery, organ, member, drug, or type of response involved. Back flow will be recorded at the site of the meter whenever the applied perfusing pressure on the central side of the meter is less than the pressure produced peripheral to the meter as the result of passive elastic recoil from the arterial tree and surrounding tissue (and the active extrinsically applied pressure in the case of the coronary arteries). Thus the greater the volume-elastic recoil component and the smaller the mean differential pressure (mean rate of flow)

at the site of the meter, the greater will be the back flow component. The rapid back flow and forward flow oscillations recorded in arteries supplying constricted beds, in contrast to the slower waves which accompany the effects of dilators, presumably are related to the higher natural frequency of the arterial bed in the constricted state.

Intravenous Drugs. An intra-arterial injection permits the transient separation of the local from the combined local and general systemic effects of a drug. An intravenous injection (via the external jugular vein) is usually followed by a variable and unpredictable admixture in time of appearance and magnitude of the two responses. Hence, the resultant changes in mean flow and flow pattern very frequently differ from those found to occur after intra-arterial injection. In many instances the vasomotor state of the bed can be established with certainty and the qualitative, if not the quantitative, change in the state is comparable to that obtained following the administration of the same drug intra-arterially. In others the early and/or intermediate (or even late) changes in the vasomotor state of the bed are not determinable from flow and pressure data.

In figure 3, E through I, are presented several series of curves which show the variability in sequence and magnitude of the flow (and blood pressure) response obtained following the intravenous injection of drugs.

In the renal artery (fig. 3E) the response to theamin can be readily interpreted and is similar to that obtained following intra-arterial injection of aminophylline (records not shown).

The intravenous administration of suprarenin causes, in the hepatic and femoral arteries (figs. 3F, H), a rise followed by a fall in mean rate of flow below the control level while blood pressure progressively rises. The later records of flow in both arteries show definite constriction but the rise in mean flow in the early records is presumably a result of an augmented blood pressure which offsets the effect of any possibly coexistent beginning vasoconstriction within the local bed.

The response in the common carotid to intravenous suprarenin (fig. 3G) is a progressive rise in mean flow and blood pressure. It is probable that the elevation in mean flow is associated with the greater driving force of an elevated systemic blood pressure which outweighs the effect of a mild constriction of the bed. However, with the recorded data alone, the possibility cannot be excluded that the bed is either unaffected by the drug, passively dilated, or even actively dilated.

A variance in dominance of peripheral and general systemic drug effects is apparent in the femoral flow curves following nitroglycerine injection (fig. 3I). With the exception of the 50 second and 9 minute records (where dilatation is present relative to the control state), mean flow and blood pressure directional changes are such that the vasomotor state of the bed cannot be identified.

SUMMARY

Optically recorded flow patterns (with an improved orifice type meter) together with the coexisting pressure pulses in the superior mesenteric, hepatic, renal,

common carotid, axillary, and femoral arteries of anesthetized dogs are presented. The effects of intra-arterial and intravenous administration of vasodilator and constrictor drugs on the flow and pressure curves are shown. Following the system of analysis previously described (1), several series of curves have been examined with reference to changes in 1, mean flow; 2, relationship of mean flow to mean pressure; 3, similarity of contour between patterns and pressure pulses; 4, volume of pulsatile deviation from the mean rate of flow (dynamic volume-elastic properties of the vascular tree), and 5, the vasomotor state of the bed (dilatation or constriction).

The generally accepted vasomotor responses to so-called dilator and constrictor drugs given intra-arterially were observed. However, in some records, and especially in those obtained following intravenous drug administration, changes in the vasomotor state of the bed could not be determined because of the overlapping of central and peripheral drug effects.

The dynamic volume-elastic component is reduced, except in the common carotid artery, by vasoconstrictor drugs given intra-arterially, while vasodilators usually increase this fraction during most of the period of drug action.

The injection of constrictor drugs, either intravenously or intra-arterially, may cause the appearance or augmentation of back flow in the patterns of all peripheral arteries so far studied. Dilators may introduce backflow in the common carotid pattern and greatly augment that preëxisting in the common carotid, axillary, and femoral arterial patterns.

The interrelationship of the determinants associated with vasomotor drug effects is considered and discussed.

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EFFECT OF pH AND CERTAIN ELECTROLYTES ON THE METABOLISM OF EJACULATED SPERMATOZOA[1]

HENRY A. LARDY AND PAUL H. PHILLIPS

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

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Within recent years there has been an increased interest in the study of the metabolism of spermatozoa. Variations in methods used in collection of semen, preparation of sperm suspensions and manometric measurements, and variations in composition of the suspension medium used in different laboratories have yielded apparently conflicting results. In order to establish optimum conditions for studying the metabolism of spermatozoa a study was undertaken to determine the influence of the ionic composition of the medium on respiration, glycolysis and motility.

METHODS. The mammalian spermatozoa used in these experiments were from normal ejaculates collected by means of an artificial vagina. The cock semen was collected from White Leghorn cocks by Dr. W. W. Cravens using a modification of the Burrows and Quinn (1) method of collection. Aliquots of the semen were accurately measured into centrifuge tubes and diluted with 1 to 3 volumes of the medium in which the sperm were to be suspended for experimentation. After centrifuging for 5 to 10 minutes the supernatant fluid was discarded. The desired amount of medium was added to each tube and the spermatozoa were suspended by drawing the medium into a 1 cc. serological pipette (at least 1 mm. inside diameter at the tip) and gently blowing it part way out. If the tip of the pipette is kept below the surface of the medium and a few millimeters above the centrifuged spermatozoa the pumping action will suspend the spermatozoa evenly throughout the medium. This process eliminates the mechanical damage, clumps or foam which may occur when stirring or shaking is employed.

As a medium for suspending the spermatozoa, calcium-free Ringer-phosphate was employed. This was prepared (with water re-distilled from glass) according to Krebs (2) except for the omission of $CaCl_2$ and was adjusted to the desired pH (glass electrode) by the addition of either 0.1 N HCl or 0.1 N NaOH. Media for studying the effects of various ions on sperm metabolism were prepared by omitting the ion in question from, or by adding the ion (in 0.154 M solution) to the Ringer-phosphate solution.

Oxygen consumption of the sperm suspension (always 3 cc. final volume) was measured at 37° in air, using either the Warburg or Barcroft apparatus. The

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central cup of each flask contained 0.2 ml. of 20 per cent KOH and a folded strip of porous filter paper to absorb CO_2. To prevent creeping of the alkali, anhydrous lanolin was spread over the rim of the central cups. The manometers were shaken at a rate of 110 oscillations per minute and a 10 to 15 minute equilibration period was allowed before the stopcocks were closed.

Lactic acid was determined by the method of Barker and Summerson (3). Motility was observed on a specimen from each flask after the experimental period as described previously (4). Where metabolites (glucose, lactate, pyruvate) were added, the final concentration in the flask was always 0.02 M.

The results are expressed according to the method of Redenz (5). Z_{O_2} = c.mm $O_2/10^8$ cells/hr. Z_L = lactic acid produced (equivalent to CO_2)/10^8 cells/hr.[2] Sperm concentrations were determined by counting in a hemacytometer. For purposes of comparison of ejaculated bull spermatozoa with other tissues the Z_{O_2} equals $2.5 \times Q_{O_2}$ (7).

We prefer to separate the spermatozoa from the seminal fluid and to study their metabolism in a medium of known composition. This eliminates the influence of metabolites in the seminal fluid as well as the endogenous respiration of the fluid itself (8). The errors involved in measuring oxygen consumption from a medium containing bicarbonate, as in the case of seminal fluid, have been discussed by MacLeod (9). The endogenous respiration has been shown by Zeller (10) to be in part due to the oxidative deamination of spermine by the enzyme diamine oxidase, and it seems likely that the oxidation of ascorbic acid may also be of importance. Bull semen contains on the average 6 mgm. ascorbic acid per 100 ml. (11) and we have found added crystalline ascorbic acid to be oxidized by seminal fluid.

Results. The effect of pH of the medium on the endogenous respiration of washed bull spermatozoa was studied over a range of pH = 6.6 to 7.5. Maximum respiration was obtained at pH = 6.9 to 7.03. With media of lower pH there was a gradual drop in respiration until at pH = 6.68 the oxygen consumption was 85 per cent of the maximum. A more rapid drop occurred on the alkaline side of optimum and at pH = 7.2 respiration was only 75 per cent of the maximum. More alkaline conditions up to pH = 7.5 did not further depress respiration. Motility was not greatly affected by variations in pH from 6.8 to 7.5 but dropped off rapidly below pH = 6.7. Although the magnitude of the respiration of spermatozoa from various bulls may vary considerably the curves plotting the effect of pH on per cent of maximum respiration were remarkably similar.

In the case of rabbit spermatozoa the optimum pH for both respiration and motility was at 6.8. This optimum was in a much narrower range than in the case of bull spermatozoa and the motility was far more sensitive to changes in pH. Oxygen consumption decreased on both sides of the optimum (75 per cent of maximum at pH = 6.0 and 7.7) but depression of motility was relatively greater on the acid side.

[2] Henle and Zittle (6) employ the symbol Z_G to designate glycolysis as measured by CO_2 replacement. Since the values in this paper are calculated from lactic acid determined by a specific chemical method the subscript L is used instead.

The optimum pH for endogenous respiration of cock spermatozoa was 7.25. Motility was equally good over the pH range studied (6.6 to 7.7) but we have found that the optimum pH for maintenance of fertility of cock sperm is near the optimum for respiration.[3]

As reported previously (7), the Z_{O_2} remains constant for sperm concentrations of 100 million to 1 billion spermatozoa in 3 cc. suspension. Henle and Zittle (6) also obtained linear respiration in this range of sperm concentration when epidid ymal secretion was added to epididymal spermatozoa.

Using suitable sperm concentrations and media of optimum pH for the species the Z_{O_2} values shown in table 1 were obtained.

Table 2 shows the effect of various ions and combinations of ions on the metabolism of bovine spermatozoa. The experiments cited are typical of two to six separate studies of the experimental variations. In each experiment calcium-free Ringer-phosphate was used as a reference for comparison.

TABLE 1

Endogenous respiration of ejaculated spermatozoa

SOURCE	NO. OF EXPTS.	Z_{O_2} (1ST HR.)	
		Range	Ave.
Bull	19	16.1–29.8	21.4
Cock	3	6.0– 7.5	6.9
Rabbit	12	7.0–16.1	10.9
Ram	4*	20.0–27.0	22.4

* pH of medium = 7.25.

All values shown were from experiments in which measurement of respiration was begun within 2 hours of ejaculation. Effect of "age" of bull spermatozoa on the oxygen consumption was studied previously (7).

Respiration was fairly good in physiological saline (expts. 1, 2, and 3) but lactic acid production was not as great as in Ringer-phosphate (expts. 1, 2, and 3). This difference was slightly greater for $Z_L^{N_2}$ values (anaerobic glycolysis). The initial motility in physiological saline was excellent but it was not maintained as well as in Ringer-phosphate Varying the phosphate level from 0 to 0.03 molar did not appreciably affect respiration but small amounts of phosphate greatly improved glycolysis in a 0.9 per cent saline medium (expt. 1). Both respiration and glycolysis were depressed in M/10 phosphate buffer (expt. 1). Addition of chloride ion improved respiration but affected glycolysis adversely indicating that perhaps osmotic relationships rather than lack of chloride ions were responsible for the inferior performance in plain phosphate buffer. Motility in Ringer-phosphate was always superior to that in M/10 phosphate buffer.

Omitting Mg^{++} from Krebs' Ringer-phosphate depressed respiration slightly. Omission of Mg^{++} depressed glycolysis but the magnitude of the depression was not consistent. Motility was usually better when the medium contained Mg^{++}.

[3] Halpin, J., C. E. Holmes, W. W. Cravens, P. H. Phillips and H. A. Lardy. Unpublished data.

Although Mg^{++} is essential for the oxidation of pyruvate by muscle (12) and brain (13), it did not increase the respiration of bull spermatozoa in the presence of pyruvate.

The addition of Mn^{++} decreased motility and anaerobic glycolysis as well as respiration and aerobic glycolysis (expt. 3).

As shown in table 3 calcium depressed both respiration and glycolysis (expt. 4) and was very detrimental to motility of bull spermatozoa. Citrate was added to one flask in experiment 5 to see if binding the Ca^{++} carried to the medium by the sperm cells would influence the respiration. Since spermatozoa do not con-

TABLE 2

Typical examples of the effect of various ions on the metabolism of bull spermatozoa

EXPERIMENT NO.	SPERM COUNT PER FLASK	MEDIUM pH = 7.0	MOLARITY OF				Z_{O_2}			Z_L^{air}
			PO_4	K^+	Mg^+	Mn^{++}	Endogenous	Glucose	Lactate	
	$\times 10^8$									
1	7.2	Ringer-phosphate	0.016	0.005	0.0012		12.0	8.4	13.5	18.3
		0.9% NaCl	0.003 to 0.03	0.001 to 0.003			11.9 12.2 ±0.5	9.4	16.7	5.7 16.0
		NaK-phosphate	0.100 0.066*	0.040 0.026			6.5	5.3 7.1	5.2	11.1 8.4
2	5.6	Ringer-phosphate	0.016 0.016	0.005 0.005	0.0012		22.3 18.6	19.5 15.7	19.8 16.1	35.1 26.3
		0.9% NaCl					14.3	15.2		25.7
3	8.2	Ringer-phosphate	0.016 0.016	0.005 0.005	0.0012 0.0012	 0.0011	16.8 15.1	17.2 14.4	16.1	19.8 16.9
		0.9% NaCl					18.4	13.5		9.4

* One cubic centimeter of M/10 phosphate buffer replaced by 0.9 per cent NaCl solution.

tain citric dehydrogenase (14–4) an increased oxygen uptake due to its oxidation would not be expected. It was found (confirmed in several other experiments) that citrate decreased respiration but *improved motility under aerobic conditions.* In experiment 6 an attempt was made to demonstrate the classical $Ca^{++} - K^+$ antagonism. In this experiment where the sperm concentration was high, the effect of Ca^{++} on respiration was not apparent but motility was greatly depressed. The effect of K^+ likewise was not appreciable in experiment 6 although it was clearly beneficial in experiment 7 and others. Experiment 6 was repeated with ram spermatozoa at pH = 7.25 and although $Ca^{++} - K^+$ antagonism was

not demonstrated, Ca^{++} had a slight detrimental effect on respiration at all levels of added K^+. A definite stimulation by K^+ of both respiration and glycolysis was obtained in experiment 7 where the spermatozoa were washed twice in K^+-free Ringer-phosphate and in other experiments where the spermatozoa were stored in the K^+-free medium for 30 minutes and then taken up in fresh media. The effect of K^+ on anaerobic glycolysis was also determined. In one experiment where the higher level of K^+ only slightly increased the oxygen consumption it more than doubled anaerobic glycolysis. In other experiments anaerobic

TABLE 3

Typical effects on bull spermatozoa of varying the K^+ and Ca^{++} concentration of the suspension medium

EXPERIMENT NO.	SPERM COUNT PER FLASK	MOLARITY OF		Z_{O_2}		Z_L^{air}
		K^+	Ca^{++}	Endog.	Glucose	
	$\times 10^8$					
4	6.3	0.005		24.8	24.1	32.7
		0.005	0.0018	18.7	13.6	29.4
		0.005	0.0037	13.3	12.3	23.8
5	7.2	0.003		17.0	11.9	
		0.003	0.0037	6.6		
		0.003*		11.5		
6	13.2			13.6		
			0.0018	13.9		
		0.006		14.4		
		0.006	0.0018	15.5		
		0.012		14.9		
		0.012	0.0018	13.7		
7†	3.7	0.000			15.1	23.0
		0.005			18.5	35.1
		0.044			22.8	33.1

Ringer-Phosphate medium used in all experiments.

* Citrate added to a concentration of 0.02M.

† Spermatozoa washed twice with K^+ -free Ringer-phosphate before making up the final suspension.

glycolysis was slightly greater when K^+ was present but the stimulation was not as great as under aerobic conditions. A potassium concentration of at least 0.005 M appeared to be essential for the best motility. Further improvement in motility with higher concentrations of K^+ was not discernible.

DISCUSSION. The calcium-free Ringer-phosphate solution which has proved so suitable for studying the metabolism of surviving tissues is likewise suitable for studying the metabolism of ejaculated spermatozoa when precautions are taken to have the pH optimum for the species. Among the ions studied, phosphate, potassium and magnesium were of importance for the maintenance of

glycolysis, respiration and motility. The observation that phosphate was apparently more important for glycolysis than for respiration might indicate that an exogenous source of phosphate is essential for glycolysis. The sperm itself must originally contain sufficient phosphate for its energy coupling mechanism since respiration and initial motility were excellent in 0.9 per cent saline. A more plausible explanation for the effect of phosphate is its buffering action. In the absence of buffer salts lactic acid produced by the spermatozoa rapidly brings the medium below pH = 6 where survival is greatly impaired.

Magnesium benefited motility and usually increased respiration but had no consistent effect on glycolysis. Potassium stimulated while manganese and especially calcium definitely inhibited all three phenomena.

SUMMARY

From this study of the metabolism of ejaculated spermatozoa in Ringer-phosphate media the following conclusions may be drawn.

1. The optimum pH values for the endogenous respiration of bull, cock and rabbit spermatozoa were 6.9 to 7.0, 7.25 and 6.8 respectively.

2. At the optimum pH for the species the endogenous respiration (measurement begun within 2 hrs. of ejaculation) of bull, cock, rabbit and ram spermatozoa was: $Z_{O_2} = 21; 7; 11$ and 22 respectively.

3. Varying the phosphate concentration of the medium from 0 to 0.03 M did not greatly affect respiration of bovine spermatozoa but glycolysis, and motility in the presence of glucose, were greatly depressed in the absence of phosphate.

4. The omission of Mg^{++} from the medium depressed respiration, glycolysis, and motility in most specimens but the effect on glycolysis was not consistent.

5. Mn^{++} and especially Ca^{++} affected motility, glycolysis and respiration adversely.

6. At least 0.005M K^{+} was necessary for maintenance of optimum motility while this and even higher concentrations stimulated respiration and glycolysis.

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BLOOD KETONE BODIES IN RELATION TO CARBOHYDRATE METABOLISM IN MUSCULAR EXERCISE[1]

A. H. NEUFELD AND W. D. ROSS

From the Research Institute of Endocrinology, McGill University, Montreal

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An important problem in the physiology of muscular exercise is the extent and manner of utilization of fat by muscles. Present evidence points to the absence of any direct rôle of fat as a muscular fuel, with the probability of its indirect contribution, possibly through the medium of ketone bodies (1, 2). Ketone bodies are removed from a perfusate by isolated diabetic muscles more rapidly if the muscles are stimulated than when they are at rest (3, 4). The situation in the intact organism somewhat obscures this change but the conflicting results (5, 6, 7) are understandable if one considers the production of ketone bodies by the liver to be increased by exercise (8). Heavy exercise over short periods of time has been shown in both rats and men to produce a drop in ketone bodies with a subsequent rise (9, 10). The evidence for the fall in ketone bodies is derived largely from one experiment in which the subject ran at 10 miles per hour for 20 minutes (11). It therefore appears desirable to place on record the results of further human experiments done in this laboratory as well as guinea pig studies in which the blood ketones were followed along with changes in carbohydrate metabolism.

METHODS. The human experiments were done on two male subjects operating a bicycle ergometer[2] against a resistance which could be varied by weights altering the tension of a brake band. The speed was kept constant by a speedometer wired in an electric bell circuit to signal if the subject was deviating from the prescribed speed. Experiments were conducted with the two subjects on a ketogenic diet when definite ketonuria was present, and on a regular balanced diet. Work was done at various measured rates of working. In some experiments work was repeated every two hours for three trials. Blood samples were taken before and after, and sometimes during the work period, and at intervals after the last trial.

The guinea pigs were run on a motor driven treadmill at 950 yards per hour. Some were sacrificed before fatigue, others at fatigue, and others after periods of recovery. The fatigue point was determined by refusal of the animal to run on stimulation by electric shock. Both fed animals and animals in ketosis from fasting for 24 hours were run. Control animals were sacrificed without running. Blood samples were taken as well as samples of muscle and liver, the latter by quickly freezing the tissues in CO_2 ice and ether.

[1] Assisted by a grant administered by the National Research Council of Canada.

[2] Appreciation is due to the Department of Physical Education, McGill, for the loan of the bicycle ergometer and to the Department of Mechanical Engineering for effecting certain alterations in this apparatus.

Analyses were made as follows: blood ketone bodies from the mercury content in Deniges' precipitate (12) expressed in terms of acetone (conversion factor 0.0633); blood pyruvate in the tungstic acid filtrate after stabilization with iodoacetate as the dinitrophenylhydrazone (13, 14); blood hemoglobin with the Evelyn photocolorimeter; lactic acid in blood and muscles by a modified Miller and Munz technique (15) after extraction of the muscles with trichloracetic acid followed by treatment with copper sulphate and calcium hydroxide; muscle and liver glycogen by the modified Pflüger method (16); sugar in blood and in the glycogen hydrolysates by Somogyi's modification of the Shaffer-Hartman method (17). With regard to the method for ketone bodies used, it was established that the amounts of lactate in the blood did not interfere.

RESULTS. *Human experiments.* On the normal diet the blood acetone varied only slightly with work, always within the normal range (0.51 to 1.25 mgm. per cent).

On the ketogenic diet initially high blood acetone levels (2 to 5 mgm. per cent, and on one occasion 14.5 mgm. per cent) showed a considerable drop (3.01 $\pm$ 0.89 mgm. per cent for 17 trials[3]) during moderately heavy work (0.097 H.P. for 15 min.) with a considerable rise two hours later (6.15 $\pm$ 1.08 mgm. per cent). During less heavy work (0.08 H.P. for 30 min.) the drop was less, but still significant (0.72 $\pm$ 0.099 mgm. per cent) and it was followed by a non-significant rise (6.84 $\pm$ 4.46). During more intense work (0.495 H.P. for 43 sec. and 0.182 H.P. for 5 min.) there was no drop.

Blood glucose during exercise showed a significant drop on a normal diet (14.33 $\pm$ 4.18 mgm. per cent for 6 estimations), but no significant drop on the ketogenic diet (1.5 $\pm$ 2.46 mgm. per cent for 12 estimations). The difference between these changes (12.83 $\pm$ 4.87 mgm. per cent) indicates a significant change.

Lactate after exercise was increased in proportion to the severity of work or in inverse proportion to the time after onset of work (73.5 mgm. after 5 min. at 0.182 H.P., 48 mgm. after 15 min. at 0.097 H.P., and 25.5 mgm. after 30 min. at 0.08 H.P.). The changes in lactate and pyruvate were similar on both diets. These are shown in figure 1, which also illustrates the typical changes in acetone and glucose in both subjects working at 0.097 H.P. for 15 min. for three trials at 2 hr. intervals.

The hemoglobin levels serve to illustrate that the changes could not be attributed to hemoconcentration or dilution. The high glucose level in D. R. about 4 hours after the last exercise on the normal diet is attributable to a meal 1 hour before this estimation. The comparable meal in A. N. was before his third trial when he showed his second highest glucose level. A. N.'s highest glucose level was 1 hour after his evening meal. Meals while on the ketogenic diet were taken at comparable times to those on the regular diet but contained very little carbo-

[3] These changes are expressed as mean and standard error. The test of significance is a ratio of mean change to its standard error (Fisher's t) such that P is less than 0.05 for significant and less than 0.01 for highly significant (18).

hydrate. Estimations made while working have been omitted from the figure in the interests of simplicity. They were generally intermediate between the before and after readings except for lactate which was sometimes higher at 5 min. than at 15 min.

Guinea-pig experiments. Some of these results are illustrated in table 1. It is of interest that the fatigue times were just as long for fasted guinea pigs as for fed ones.

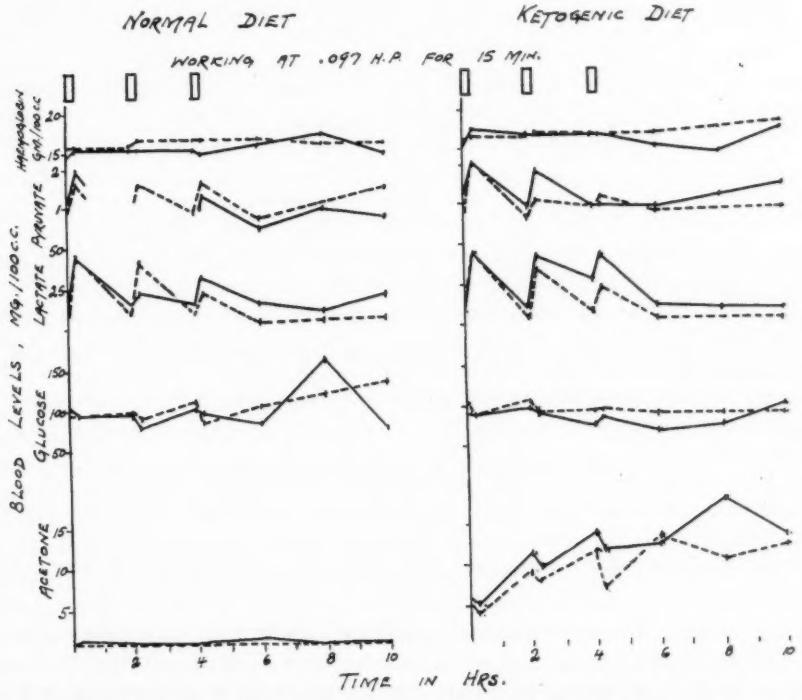

Fig. 1. Blood changes in two human subjects while working on a bicycle ergometer and during the subsequent recovery period (subject D. R., continuous line; subject A. N., broken line).

The significant differences in the fasted animals from the fed ones are as follows: initial blood acetones higher ($t = 3.65$) and muscle and liver glycogen lower ($t = 4.17$ and 13.0); rise in blood acetones at fatigue greater ($t = 3.83$); rise in blood lactate at 10 min. less ($t = 3.11$); drop in muscle and liver glycogen less ($t = 3.47$ and 9.70). The slightly lower blood acetone at 10 min., the slightly higher blood glucose at fatigue and the slightly less drop in blood glucose in the fasted animals are not significant, although they tend in the direction of the findings in the human experiments.

Results from animals sacrificed at other times previous to fatigue and after various periods of recovery have been omitted from the table to avoid complexity. These results indicated that the peak of lactate and pyruvate level was at around 10 min. of working and that this was followed by a lower plateau previous to a second rise at fatigue. There was an appreciable rise of ketone bodies during recovery.

TABLE 1

Blood and tissue changes in guinea pigs while running on a motor driven treadmill

	FED			FASTED		
	Control	10 mins.	Fatigue	Control	10 mins.	Fatigue
	No. of animals					
	7	7	5	4	3	3
	Time to fatigue (min.)					
	184 ± 24			219 ± 25		
Blood						
Acetone (mgm./100 cc.)	0.99 ± 0.114	1.41 ± 0.163	9.07 ± 1.24	2.77 ± 0.475	1.76 ± 0.38	29.90 ± 2.82
Sugar (mgm./100 cc.)	126.7 ± 6.10	128.1 ± 4.85	60.6 ± 2.05	111.5 ± 6.39	128.6 ± 4.63	7.20 ± 8.08
Lactic (mgm./100 cc.)	18.1 ± 1.66	64.8 ± 9.93	46.7 ± 9.65	13.5 ± 1.40	33.65 ± 1.25	26.2 ± 2.5
Muscle						
Lactic (mgm.%)	23.7 ± 2.08	117.4 ± 11.72	61.5 ± 12.24	22.86 ± 3.64	76.18 ± 18.62	38.7 ± 9.44
Glycogen (mgm.%)	923 ± 70.4	596 ± 59.0	168 ± 19.0	584 ± 17.3	438 ± 32.2	102 ± 24.8
Liver glycogen (%)	5.79 ± 0.42	5.67 ± 0.52	0.38 ± 0.17	0.17 ± 0.10	0.14 ± 0.08	0.033 ± 0.009

Discussion. The performance of moderate work by the two human subjects studied while on a normal diet was associated with a significant drop in blood glucose level. One may assume that this was the result of an increased flow of glucose into the muscles to replenish the glycogen stores. Continued moderate work would thus entail a decreasing liver glycogen level as a result of the change: liver glycogen → muscle glycogen → lactic acid. This appears to be substantiated in the observations that the fed guinea pigs, following exhaustive muscular exercise, had lost almost their entire supply of liver glycogen. This strongly suggests that in the presence of sufficient carbohydrate stores the oxidation of lactic acid during muscle contraction proceeds at a rapid rate. As a matter of fact, Dill and his associates (19) were able to demonstrate a dependence of the rate of lactic acid removal on the rate of metabolism. The changes in blood pyruvate, a substance known to be an intermediate in the breakdown of carbohydrate still further supports this view.

The performance of moderate work by the human subjects while on a ketogenic diet was not associated with a drop of blood glucose. It was associated, however,

with a significant disappearance of blood ketone bodies. Following cessation of work blood ketone bodies increased, presumably as a result of the increased production of these substances by the liver, associated with the sudden stop of utilization. The results obtained with the fasted guinea pigs are essentially the same. The small discrepancies between the findings in the humans and the guinea pigs can be accounted for by the fact that the humans, although in ketosis, were not fasted and may have had larger glycogen stores as compared to their own controls than did the fasted guinea pigs.

In the human experiments the more strenuous exercise for 45 sec. and for 5 min., which produced the greatest increase in lactate, did not result in any decrease in blood ketone bodies. A portion of the energy expanded was probably supplied anaerobically and the muscles were dependent on the "lactic acid mechanism" for contracting in an oxygen debt. Presumably this, together with the available lactic acid, was sufficient to meet the energy requirements.

SUMMARY

A study has been made on human subjects while on normal and on ketogenic diets, on the effect of work (bicycle ergometer) on the blood ketone bodies, glucose, lactate, pyruvate and hemoglobin. A similar study has been made on fed as well as fasted guinea pigs running on a motor driven treadmill. In these, muscle lactate and muscle and liver glycogen changes also were followed.

Pedalling at 0.097 H.P. for 15 min. while on a ketogenic diet produced a highly significant drop in blood ketone bodies (3.01 $\pm$ 0.89 mgm. per cent). At the same time the drop in blood glucose which was shown on a normal diet failed to occur. Cessation of work resulted in a significant increase in ketone bodies. Results were obtained with the fasted guinea pigs, which illustrated a lower utilization of carbohydrate stores for the same amount of work than in the fed ones. It appears from the results reported that ketone bodies can be utilized by muscles and that during exercise their rate of production by the liver is increased. This, however, becomes apparent only during deficiency of carbohydrate stores (fasting, or a ketogenic debt).

We wish to thank Prof. J. B. Collip under whose direction this work was carried out.

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THE EFFECT OF HEMORRHAGE ON NORMAL AND HYPOCOAGULABLE BLOOD AND LYMPH

B. G. P. SHAFIROFF, H. DOUBILET, R. SIFFERT AND CoTUI[1]

From the Laboratory of Experimental Surgery, Department of Surgery, The New York University College of Medicine

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The effect of acute hemorrhage on the animal whose blood and lymph are hypocoagulable has not been previously tested, nor has the relationship to the blood of either normal or hypocoagulable lymph been adequately determined. However an increased acceleration in the coagulation time of the blood during rapid progressive hemorrhage has been noted by many observers (1) (2). Although the cause for this change has not been fully established, Drinker et al. have suggested that the lymph might be the responsible contributory factor in this altered coagulability of the blood (3).

In the present investigation, the effect of acute hemorrhage was studied in dogs whose blood was rendered hypocoagulable by a variety of anticoagulants. The plan was to determine quantitatively changes in the clotting time and in the coagulation factors in samples of blood and lymph taken at successive intervals during the course of hemorrhage.

Twenty normal dogs were divided into four groups and were prepared in the manner described below.

Group I. Five dogs were subjected to hemorrhage and lymph drainage.

Group II. Five dogs were given an intravenous infusion of 50 cc. of 1 per cent protamine solution (salmine sulfate) over a period of 15 minutes and then subjected to hemorrhage and lymph drainage.

Group III. Five dogs were injected intravenously with 50 cc. of 20 per cent Witte's peptone solution and were then subjected to successive bleedings and lymph drainage.

Group IV. Five dogs were given an intravenous injection of heparin (10 mgm. per kilo) and subjected to hemorrhage and lymph drainage.

Each dog was anesthetized by receiving an intravenous injection of 1 cc. of 3 per cent pentobarbital sodium per kilo of body weight. The thoracic lymph duct was isolated in the neck, dissociated from its entrance into the subclavian vein, and intubated with a Lindemann needle. Rapid successive bleedings were effected by the withdrawal of 45 cc. of blood from the femoral artery at 20 minute intervals. Lymph was continuously collected and also sampled at 20 minute intervals. One and a half cubic centimeters of $2\frac{1}{2}$ per cent sodium citrate solution was added to each 10 cc. of blood or lymph. In addition for each of the groups described above, two dogs serving as controls were studied under identical conditions but were subjected to the removal of only 5 cc. of blood and lymph at 20 minute intervals during the experimental period. The following deter-

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minations were made on each sample of blood and lymph; coagulation time (Lee and White method) (4), plasma coagulation time (Howell recalcification method) (5), prothrombin time (Quick method) (6), antithrombin time (7), and fibrinogen concentration (8).

RESULTS. *Group I.* The coagulation time of the blood and lymph usually decreased after each withdrawal of blood. As a result of the repeated bleedings, the clotting time of the blood fell an average of 106 seconds below its initial value, and the clotting time of the lymph was reduced an average of 80 seconds. The coagulation time of the plasma from the blood and lymph followed the same trend as the latter. The prothrombin time and the antithrombin time showed no significant changes in either the blood or lymph. After the first three or four bleedings, the concentration of fibrinogen tended to rise in the blood and lymph but fell markedly as the experiment progressed. In the control dogs for this group, there were no significant changes in the coagulation factors or in the clotting time of either the blood or lymph.

Group II. After the completion of the injection of the protamine solution, the coagulation time of the whole blood and lymph was markedly prolonged, remaining incoagulable for more than four hours. At the end of the experiment, the coagulation time of the blood was reduced to about 10 minutes while the post-injection samples of lymph stayed hypocoagulable. The plasma coagulation time of the blood was increased markedly above its initial value and then fell progressively, while the plasma coagulation time of the lymph remained elevated throughout the entire period of hemorrhage. Prothrombin time and antithrombin time which were prolonged in the blood and lymph after the injection of protamine declined slowly toward their initial values. Also as a result of the injection, there was a marked reduction in the fibrinogen concentration of the blood and lymph which continued at a low level during the course of the experiment. Noteworthy was the rapid and profuse flow of lymph which was induced by the injection of protamine and was not affected by the rapid bleedings which served to slow the flow of lymph in the dogs of group I. In the control dogs, the prolongation of the coagulation time, the prothrombin time, and the antithrombin time of the blood and lymph persisted throughout the entire period of observation with but moderate subsidence of the anticoagulant effect of protamine. The concentration of fibrinogen decreased quantitatively in the blood and lymph of the control animals even though they were not subjected to hemorrhage.

Group III. The intravenous injection of Witte's peptone produced an effect on the clotting time of the blood and lymph and their coagulation factors similar to that produced by protamine. Except for the failure of the coagulation time of the lymph and its plasma to fall below their post-injection values, the clotting time of the blood and the blood plasma fell markedly as a result of the bleedings so that the final values were lower than the initial values and comparable to the effect of hemorrhage in the dogs of group I.

Group IV. After the intravenous injection of heparin, the clotting time, the plasma coagulation time, the prothrombin time and the antithrombin time of

the blood were increased. Heparin acted more directly on the blood and did not appear to affect significantly the flow of lymph or its coagulation factors. Also, the concentration of fibrinogen in the blood and lymph was not markedly affected by the injection of heparin. After repeated bleedings, the final coagulation time of the blood and lymph were reduced below their original values. In the control dogs, the heparin effect on the coagulation factors of the blood and lymph was similar to that described above but subsided significantly as its anticoagulant action wore off. However, the changes were not as marked as in the heparin dogs subjected to hemorrhage.

Discussion. It may be assumed that an increase in concentration of thromboplastin in the plasma was responsible for the hypercoagulability of the blood following hemorrhage, since the responsible factors were apparently neither

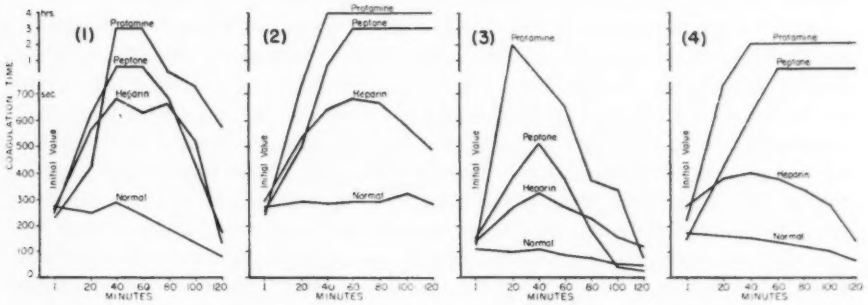

Fig. 1 shows the changes in the coagulation time (ordinate) of normal and hypocoagulable blood (protamine, peptone or heparin) during rapid progressive hemorrhage. Figure 2 shows the changes in the coagulation time of normal and hypocoagulable blood in control dogs not subjected to hemorrhage. In contrast with figure 1, the acceleration of the clotting time as a result of hemorrhage does not occur. Figure 3 shows the changes in the coagulation time of normal and hypocoagulable blood plasma during hemorrhage. Plasma coagulation time and the clotting time of the blood parallel each other. Figure 4 shows the effect of hemorrhage on normal and hypocoagulable lymph during the course of the experiment. Only the clotting times of the lymph of the normal and heparin injected animals follow the same trend as the clotting time of the blood.

prothrombin, antithrombin or fibrinogen. It may be assumed also that the increased mobilization of thromboplastin was due to a more rapid lysis of platelets (9) as one of the compensatory mechanisms of hemorrhage. The resultant reduction or acceleration of the coagulation time was manifested both in the blood and the plasma during the course of rapid progressive hemorrhage.

The mechanism by which the anticoagulants suppress the coagulability of the blood has not been fully explained. Some authors have claimed that each anticoagulant retards the clotting of blood in a specific manner; i.e., protamine by inactivating thromboplastin by forming a cephalin-protamine complex (10), peptone by increasing the antithrombic titre in the blood (11), and heparin by its antiprothrombic or antithrombic activity (12). Another group of observers have indicated that anticoagulants such as used in these experiments cause a

mobilization of heparin in the blood which inhibits the lysis of platelets thus preventing the liberation of thromboplastin and retarding coagulation (11). However, regardless of the method of inhibition of the coagulability of the blood, the response of the clotting mechanism to acute hemorrhage was usually the same as in the uninjected animals of group I, namely, an acceleration of the coagulation time and a trend toward hypercoagulability. The controls in which the animals were not subjected to hemorrhage and in the others whose blood was rendered hypocoagulable failed to show this response.

The results of these experiments support similar clinical observations made by Sahli (13) who observed that hemorrhage in hemophiliacs resulted in a reduction of the prolonged coagulation time of the blood, and by Lawson and Graybeal (14) who found that by the routine therapeutic withdrawal of blood, the frequency of hemorrhages in a hemophiliac was significantly decreased.

Although no evidence was found to indicate a special function or contribution of the thoracic duct lymph to the coagulating mechanism of the blood, other equally interesting observations were made. The clotting time and the values of the coagulation factors of the blood and of the thoracic duct lymph differed quantatitively and did not indicate that any relationship existed between the two on a purely filtration basis. During the course of the hemorrhage experiments, the coagulation time of the lymph in the normal and heparin injected animals followed the same pattern as that of the blood, while in the protamine and peptone series, the lymph remained hypocoagulable in spite of hemorrhage and the reduction of the clotting time of the blood. The latter hypocoagulability may be accounted for on the basis of a dilution effect since the rate of flow and the quantity of lymph were greatly increased. In the present experiments, it was possible to obtain a hypocoagulable blood (heparin group) without markedly affecting the clotting time of the lymph, and Shore (15) showed that it was also possible to render the lymph hypocoagulable without affecting the coagulability of the blood.

SUMMARY

1. Rapid progressive hemorrhage rendered the blood and lymph of normal animals hypercoagulable.

2. Rapid progressive hemorrhage also caused a marked reduction of the clotting time in animals whose blood was experimentally rendered hypocoagulable.

3. The mechanism for increased coagulability of the blood during hemorrhage was related to the increased mobilization of thromboplastin.

4. The lymph of protamine and peptone injected animals remained hypocoagulable in spite of hemorrhage.

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DRUG ACTIONS ON THE SPONTANEOUSLY BEATING TURTLE VENTRICLE INDICATING LACK OF INNERVATION*[1]

EDWIN P. HIATT AND WALTER E. GARREY

From the Physiology Laboratory of the Vanderbilt University School of Medicine, Nashville, Tennessee

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Since the pronouncement of Gaskell (1900), evidence tantamount to proof has accumulated that the turtle ventricle is not innervated by inhibitory vagus fibers. The relations of the sympathetic nerves to this tissue are not equally clear. In this report we offer evidence to indicate that the turtle ventricle is without either sympathetic or parasympathetic nerves.

Garrey and Chastain (1937) have already shown that eserinized ventricular strips from the turtle heart, caused to contract by regular electrical stimuli, show no inhibition when treated with acetylcholine. They suggested that this indicated a lack of cholinergic nerves to the tissue. We have repeated this observation on ventricular strips beating spontaneously. We have also tested such automatically active strips with a number of the so-called autonomic drugs, sympathomimetic as well as parasympathomimetic, without obtaining any evidence of neural control.

METHODS. Thin strips of turtle ventricle, usually from the apical half, were suspended in a cup containing a physiological saline so that their contractions were recorded on a kymograph. Oxygen was bubbled vigorously through the solution during the whole time of observation. As Howell (1901) has reported, these ventricular strips do not, as a rule, beat spontaneously in the animal's own serum or in an equivalent salt solution. He has indicated that this is due to the concentration of potassium present. In our physiological saline for turtles, which contains considerably less potassium than most turtle serum, according to the analyses of the latter made by Smith (1929), the strips usually began contracting soon after they were immersed. This turtle saline consists of 120 mM NaCl + 2.5 mM KCl + 2.5 mM $CaCl_2$ per liter of water alkalinized to a pH of approximately 7.8 with $NaHCO_3$. Some strips contracted regularly from the beginning, but most contracted irregularly. They could be caused to beat regularly by transferring them to a solution containing less potassium or by soaking them in the saline in the refrigerator for several hours. Strips left overnight or for several days, immersed in this saline, usually beat regularly. We have occasionally used strips which had been seven days in the refrigerator.

Drugs were added in alkaline solution to the bath where they were evenly distributed by the stirring action of a stream of oxygen.

Usually the auricles from the same heart were also suspended in the bath to record separately the action of drugs on innervated tissue and thus control drug

[1] Aided by a grant from the Bristol-Myers Company.

potency as well as other conditions. It should be explained that the strips of ventricle are not only thin but also spongy in structure, so that materials can diffuse into them as easily as they enter the auricular tissue.

Finally the auricles and the ventricle of the frog heart were suspended separately in the same solution and subjected to the action of the same drugs. The ventricle of the frog is known to have both sympathetic and parasympathetic nerve supply to the myocardium. Its reactions to autonomic drugs are, in contrast to those of the turtle ventricle, like those of the turtle auricle. This adds convincingly to the evidence indicating the turtle ventricle to be without autonomic nerves to the myocardium.

RESULTS. *Acetylcholine.* After being treated with eserine (usually 1:1,000,000), the tissues were exposed to acetylcholine in concentrations up to 1:10,000. In concentrations several times greater than those required to completely inhibit the auricular beat, there was no inhibition of the ventricular beat, which was unaffected (fig. 1). The frog ventricle was inhibited by acetylcholine in the same manner as the auricle (fig. 2).

Pilocarpine. In concentrations far in excess of those which cause complete cessation of beat in the auricle, pilocarpine had no effect on the ventricular beat (fig. 1). The frog ventricle again was inhibited in the same manner as the auricle (fig. 2).

Adrenaline. The ventricular strips were treated with adrenaline in various concentrations from 1:25,000,000 to 1:10,000. In concentrations which cause a maximal acceleration of the turtle heart and an increase in the amplitude of its auricular beat, adrenaline caused only a slight increase in the amplitude of the ventricular contraction without any marked increase in rate (fig. 1). This increase in amplitude varied in different preparations from nothing at all to a maximum of 15 per cent, whereas the auricles and the frog ventricle (fig. 2) will often show an increase in amplitude of 100 per cent. The effect on the turtle ventricle decreases with time after removal from the animal. Furthermore, it is difficult to get the adrenaline effect repeatedly on the turtle ventricle, since the increase in amplitude is not so readily reversed in fresh saline as it is in the auricles and in the frog ventricle. All this suggests that the ventricle of the turtle does not react to adrenaline as do the turtle and frog auricles and the frog ventricle because it does not possess sympathetic nerves nor the receptor substance which accompanies innervation. This would mean that the slight increase in amplitude which the ventricular contractions show must be due to a direct effect on the cell, in the sense that it does not require a special reaction at the neuromuscular junction.

Ephedrine. Ephedrine had the same action as adrenaline (figs. 1 and 2).

Potassium excess. The beat of a fresh ventricular strip is stopped by a very small excess of potassium but strips which have been kept for some time in saline in the refrigerator are much more resistant to the action of potassium, becoming more like the auricles and the frog ventricle in this respect. This action of potassium can be quickly reversed on returning to the original solution. The effect is not on the contractile mechanism because the beat stops abruptly without any

weakening of the contraction, and the occasional beats which occur after the rhythm has been stopped are usually of normal amplitude. Furthermore, one of us (W. E. G.) has unpublished records showing that the electrically stimulated ventricle is not thus arrested by low concentrations of potassium of this order but instead behaves like the auricle, showing a gradual decrease in amplitude with increasing potassium chloride concentrations beginning around 100 mgm. per cent. The effect of potassium on the spontaneously contracting strips is there-

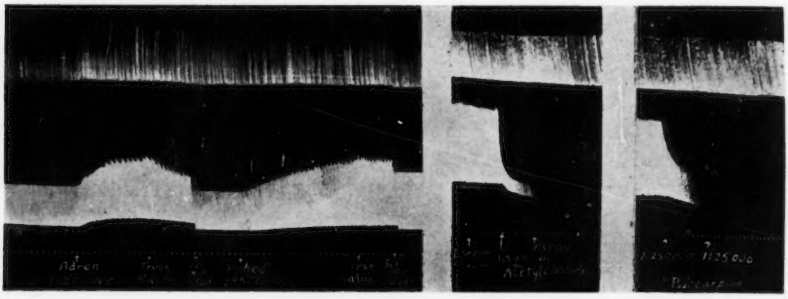

Fig. 1. Kymograph tracings of the contractions of strips of the auricle and the ventricle from the turtle heart showing the effects, from left to right, of adrenaline (1:5,000,000), ephedrine (1:500,000), acetylcholine (1:5,000,000 and 1:50,000) after eserine, and pilocarpine (1:250,000 and 1:25,000). Time = 30 sec. intervals.

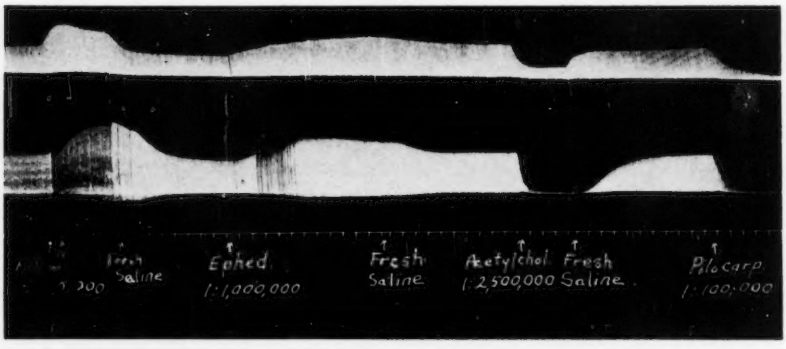

Fig. 2. Kymograph tracings of contractions of the auricles and the ventricle of the frog heart showing, from left to right, the effects of adrenaline (1:5,000,000), ephedrine (1:1,000,000), acetylcholine (1:2,500,000) and pilocarpine (1:100,000). The upper tracing was made by the ventricle, the middle tracing by the auricles and the bottom tracing by the time marker indicating intervals of 30 sec.

fore to stop the pacemaker reaction or to prevent the conduction of the stimulus to the contractile mechanism. Adrenaline and calcium can counteract to some degree these effects of potassium. In this connection it should be pointed out that acetylcholine and potassium, which are similar in so many of their effects, differ markedly in their action on these ventricular strips and hence in the mechanism by which their effects are produced.

Calcium excess. Excesses of calcium cause an increase in the amplitude of the contraction of both auricle and ventricle in the turtle as well as in the frog; in accordance with our hypothesis this represents an effect directly on the cell.

DISCUSSION. Studies on tissues without nerve supply give promise of adding to our knowledge of the site of action of the autonomic drugs, particularly in the gap between the production of chemical mediators at nerve endings and the specific response of the effector. This gap is at present filled only with the term "receptor substance." Unfortunately the reports of work in this field have not always shown agreement. (See Euler (1938) and Armstrong (1935) for literature.) The gist of the matter seems to be that whereas non-innervated tissue (embryonic heart, placental blood vessels, etc.) may react to autonomic drugs, they usually do so only at high concentrations. It seems certain that innervation confers a special sensitivity to these drugs, which may be due to the development of a receptive substance at the neuromuscular junction. This sensitivity, as is well known, persists after the nerves have been sectioned and have degenerated, so it must be intimately connected with the muscle cells. (Cannon and Rosenblueth, 1937.)

Of the agents we tested, adrenaline, ephedrine, potassium and calcium affected the contraction of the turtle ventricle in low concentrations. If our evidence mentioned above be accepted as proof of the lack of innervation in this tissue, then these agents can be considered as having a direct action on the myocardial cells in the absence of any specific receptor substance associated with innervation. Adrenaline and ephedrine, however, have a much greater effect in the presence of innervation, whereas the effect of potassium in stopping the beat was most evident on the non-innervated turtle ventricle.

SUMMARY

1. Isolated strips of the ventricle of the turtle heart will beat spontaneously and regularly if bathed in a well oxygenated physiological saline solution containing potassium and calcium in equivalent molar concentration but having less potassium than turtle serum.

2. These ventricular strips do not show inhibition either of impulse initiation or contractility when treated with acetylcholine (plus eserine) or pilocarpine in concentration far in excess of those required to inhibit the auricle and the frog ventricle. This confirms previous work indicating that the turtle ventricle lacks a vagus nerve supply.

3. Adrenaline and ephedrine cause a small increase in the amplitude of the contractions of ventricular strips but the effect is quite small compared with the effect on the auricle and on the frog ventricle. It is suggested that this indicates a lack of sympathetic nerve supply.

4. A small excess of potassium, which has little effect on the auricle or the frog ventricle, causes cessation of the beat of fresh turtle ventricle strips. The effect is not on the contractile process. The sensitivity of these strips of turtle ventricle to potassium decreases with time after excision.

5. Calcium excess causes an increase in the amplitude of both auricular and ventricular contractions.

6. It is indicated that acetylcholine, pilocarpine, adrenaline and ephedrine have their principal effects on the heart muscle only through the receptor substance which accompanies innervation. Adrenaline and ephedrine have a slight stimulating action on the aneuric myocardial tissue. Potassium and calcium appear to have a definite action directly on the myocardial cells lacking the receptor substance.

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RESPONSES IN SIZE, OUTPUT AND EFFICIENCY OF THE HUMAN HEART TO ACUTE ALTERATION IN THE COMPOSITION OF INSPIRED AIR[1]

ANCEL KEYS, J. PAUL STAPP AND ANTONIO VIOLANTE

From the Laboratory of Physiological Hygiene, University of Minnesota

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Cardiovascular responses to acute hypoxia have been extensively studied but fundamental data from rigidly controlled experiments on man are still not numerous. It is well known that sudden change to oxygen partial pressures of 90 mm. Hg or less causes acceleration of the pulse which is increasingly marked with decreasing pO_2. A bluish pallor and cold extremities are noted. When persons are maintained in this state for some minutes or hours they may suddenly collapse in syncope with an almost imperceptible pulse or they may develop marked complaints of dyspnea, "pounding heart," and violent headache. Occasional "heart attacks" and even sudden death in "normal" people at high altitude are sometimes mentioned.

It is not surprising, therefore, that it is frequently believed hypoxia imposes a severe strain on the heart and that many of the important symptoms of hypoxia are referable to either cardiac insufficiency or to an adaptive hypertension. In support of this view is the fact that cardiac dilatation is readily produced by anoxia in isolated hearts and in acute animal experiments and that severe exertion plus the chronic hypoxia of high mountains is reported to produce dilatation of the heart in man (Van Liere, 1927; Spycher, 1931).

It should be noted, however, that critical data on man are absent or extremely meager for: 1, the size of the heart; 2, the stroke output and minute output; 3, the relative efficiency of the heart in the hypoxia condition. Studies on these and related questions on 27 normal subjects are reported here.

PROCEDURE. Healthy male subjects between the ages of 18 and 30 years were studied. All were free from signs of cardiovascular abnormality. All experiments were made in a constant environment room (78°F., humidity 45 per cent to 55 per cent relative saturation). The subjects were quietly seated in front of the roentgenkymograph throughout the experiments.

The subjects breathed through an anesthesia mask supplied with, successively, room air, the test gas mixture, and room air. Krogh type low-resistance diaphragm valves were used. The total respiratory dead space of the apparatus system was 160 cc. Supply and collection gasometers were well-balanced and compensated. In most cases where syncope did not intervene the test gas mix-

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ture was inspired for 15 to 20 minutes (average 17 min.), but in some experiments the periods were shorter (10 to 14 min.) or longer (24 to 48 min.). The initial control and final recovery periods were usually 20 to 30 minutes each.

The partial pressures of oxygen used in the test gas mixtures corresponded to altitudes of 18,000 to 28,000 feet. In some cases carbon dioxide at partial pressures of 14 to 30 mm. Hg was added to the inspired air. In another series of experiments pure oxygen was supplied as the test gas mixture.

Measured variables. Heart rate, blood pressure and respiration were measured at very frequent intervals by recording devices and a team of three observers. Roentgenkymograms were taken 1, immediately before throwing the valve from room air to the test gas; 2, during the last 2 minutes of respiration of the test gas, and 3, after 10 to 25 minutes of recovery. Cardiac volumes in diastole and in systole and stroke outputs were measured and calculated according to the methods developed in this Laboratory (Keys et al., 1940).

Electrocardiographic findings, oxygen consumption, respiratory quotient, and so on were measured in many experiments. None of the latter appeared to provide useful new information and accordingly will not be discussed here in any detail.

Derived variables. Minute volume output of the heart was calculated from the stroke output and the average pulse rate during the minute in which the roentgenkymographic exposure was made.

An index of "*relative cardiac work*" was obtained from the product of the minute volume and the mean blood pressure characteristic of the period in which the minute volume was measured. Similarly, an index of "*relative cardiac effort*" was obtained from the product of the heart rate and the diastolic volume of the heart.

An index of "*relative cardiac efficiency*" is supplied by the ratio of "work" to "effort." Since we are concerned in changes rather than absolute values or comparisons of individuals, such indices should be acceptable if the values in the test period are expressed as percentages of the control values on the same individual.

RESULTS. *Simple hypoxia.* A number of the experiments with simple hypoxia were terminated by the sudden collapse of the subjects. Since these require separate consideration we shall discuss first the experiments in which collapse did not occur.

Blood pressure and pulse records from a typical experiment are given in figure 1. In this experiment the diastolic volume after 13 minutes of hypoxia was 98 per cent of the control volume and the minute volume was 130 per cent of the control. The stroke output was almost exactly the same in both periods. In 15 experiments of this kind with pO_2 between 60 and 80 mm. Hg (average 75 mm.) the pulse rate increased in 10 to 20 minutes from 10 to 28 beats per minute (average +19). There was no consistent change in systolic blood pressure (range −12 to +18 mm. Hg), but the diastolic pressure declined 6 mm. on the average (maximum −24 mm.). The pulse pressure was increased in 12 out of the 15 experiments (average +7 mm., maximum +28 mm.). The minute

volumes, diastolic heart volumes and the ventilations are tabulated, together with the calculated "work," "effort" and "efficiency" in table 1.

It appears that cardiac "work" and "effort" are increased by about 25 per cent on the average, that this is achieved with no increase in diastolic heart size and that cardiac "efficiency" is certainly not impaired. It is interesting that, on the average, ventilation and minute volume are increased to very nearly

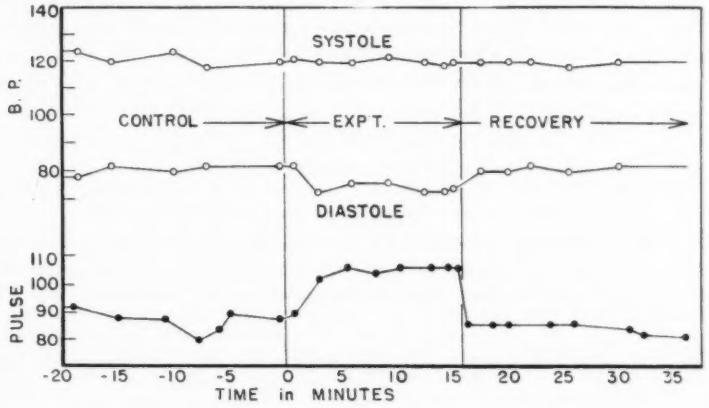

Fig. 1. Blood pressure and pulse rates in a typical low-oxygen experiment (expt. C1-29). During the experimental period the oxygen content of the inspired air was 8.96 per cent. Relative diastolic volume of the heart was 100 at −2 minutes and 98 at +13 minutes. The stroke output of the heart was identical at both of these times.

TABLE 1

Mean changes induced by hypoxia in minute output and diastolic volume of the heart, ventilation of the lungs, and in "work," "effort," and "efficiency" of the heart

All values are for inspiration of air with oxygen at a partial pressure of 64 to 79 mm. Hg and are expressed as percentages of the control levels measured immediately prior to the induction of hypoxia. Barometric pressure averaged 740 mm. 15 experiments.

	PER CENT O_2 INSPIRATION	MINUTE VOLUME	VENTILATION	DIASTOLIC VOLUME	"WORK"	"EFFORT"	"EFFICIENCY"
Mean	10.4	137	132	100	128	123	104
Standard deviation		±31	±24	±6	±20	±8	±15

the same extent—about 35 per cent. In all cases the several variables returned to approximately the initial control levels in 1 to 5 minutes after the resumption of breathing ordinary room air.

Hypoxia with syncope. The recognition of syncope and the timing of its appearance is somewhat difficult in these experiments. Dimness of vision and auditory confusion frequently appeared as did stupor bordering on unconsciousness. In 10 experiments complete anoxic syncope with postural collapse occurred within 2.8 to 15 minutes of the start of breathing the low oxygen

mixture. In these cases there was frequently twitching of isolated muscle groups and slight uncoordinated and apparently involuntary movements of the extremities. These signs were also seen in several instances in which collapse did not occur.

Collapse set in abruptly with few premonitory signs. The blood pressure suddenly fell to zero on the sphygmomanometer and the heart beat became almost imperceptible. Immediately prior to this the pulse was always strong and regular. In several instances sudden hypertension developed just before collapse but this was the exception rather than the rule. In most cases the pulse rate in the hypoxic period before collapse was more rapid than in experi-

TABLE 2

Anoxic syncope

Blood pressures and pulse rates immediately before and in recovery from anoxic syncope induced by respiration of oxygen at partial pressure of 45 to 78 mm. Hg

"Syncope time" represents minutes after start of low oxygen when definite syncope occurred. Values "Before Syncope" were recorded within 30 seconds of collapse. Values in "Recovery" were recorded from 2 to 4 minutes after return to room air.

EXPERIMENT NUMBER	O_2	SYNCOPE TIME	MEAN CONTROL		BEFORE SYNCOPE		RECOVERY	
			Pulse	Blood pressure	Pulse	Blood Pressure	Pulse	Blood pressure
	per cent							
3	9.70	15	92	110/86			60	90/70
4	9.70	6.5	76	120/84	106	118/78		
6	10.68	6	78	118/90	94			
21	10.41	6.5	84	108/80	120	108/76	80	114/84
23	10.78	6.5	85	116/80	105	116/66		
40	6.18	2.8	74	122/88	120	145/74	62	125/75
41	6.18	4	82	122/84	140	175/70	64	125/80
42	6.18	3.5	84	122/80	(120)	186/84	(60)	136/80
46	7.75	4	95	120/88	120	112/80	69	120/88
47	7.75	3	80	124/92	118	114/88	60	92/74
Mean...	8.53	5.8	83	118/85	116	134/77	65	114/79

ments where collapse did not occur. Prior to collapse the respiration became shallow and irregular and the appearance was of respiratory failure as the primary cause of collapse.

Naturally it was not possible to obtain roentgenkymographs just before or at the moment of collapse, but any dilatation, if it occurred, had regressed to normal when exposures were made in several cases a few minutes later. It is interesting to note that in all cases there was a relative bradycardia in the early stages of recovery. On recovery none of the subjects seemed any the worse for their experience. There was no memory of events leading to the collapse and shortly thereafter. Data on these experiments are summarized in table 2.

Hypoxia with carbon dioxide. The medical use of carbon dioxide to improve respiration when there is actual or incipient hypoxia is now well established.

Part of the efficacy of respirator systems like the B-L-B mask may be ascribed to the elevation of pCO_2 in the inspired air. The effect of carbon dioxide at a partial pressure of 16 to 24 mm. Hg with oxygen at pO_2 from 56 to 81 mm. was studied in 12 experiments.

The addition of carbon dioxide resulted in marked improvement of the subjects compared with similar or somewhat higher partial pressures of oxygen alone. Syncope only occurred in one experiment. Even in this one case postural collapse was not complete but there were involuntary movements and loss of consciousness after 9 minutes of respiration of 8.55 per cent O_2 plus 2.63 per cent CO_2. In this case the pulse rate rose to 104 from a control level of 84 and the blood pressure suddenly rose in one minute from 132/90 to 160/98 and then subsided to 104/70 at the onset of signs of syncope.

In the other subjects there was a moderate increase in heart rate but the

TABLE 3

Mean changes induced by hypoxia plus carbon dioxide in output and diastolic volume of the heart, ventilation of the lungs, and in "work," "effort," and "efficiency" of the heart

All values are expressed as percentages of the control levels measured immediately prior to the induction of hypoxia. Barometric pressure averaged 740 mm.

EXPERIMENT NUMBER	O_2	CO_2	VENTILATION	DIASTOLIC VOLUME	MINUTE VOLUME	"WORK"	"EFFORT"	"EFFICIENCY
	per cent	*per cent*						
18	9.9	3.34	204	97	110	111	104	107
19	10.0	2.38	178	100	119	130	118	110
22	10.6	2.44	154	94	105	110	104	106
25	11.3	2.22	166	96	103	108	114	95
26	9.5	2.77	192	99	103	111	105	105
28	9.6	2.04	154	99	145	123	111	111
Mean...	10.06	2.63	175	97.5	114	115.5	109.2	105.7

blood pressure changes were small. The subjects remained much more alert and responsive than with simple hypoxia. All of them noticed their increased ventilation which averaged 78 per cent greater than the control level. Several of these same subjects collapsed when breathing oxygen at a slightly higher partial pressure but without any carbon dioxide.

Roentgenkymographic studies were made in 6 of these CO_2 plus hypoxia experiments. The changes of minute volume output of the heart were smaller than in simple hypoxia. There was no change in diastolic heart volume. Both cardiac "work" and "effort" were increased in all cases but to a smaller extent than in simple hypoxia. "Efficiency" was undiminished.

In 6 control experiments CO_2 was administered at partial pressures of 23 to 28 mm. Hg in ordinary air and in oxygen-CO_2 mixtures. The ventilation increased 79 per cent on an average (range +44 per cent to +100 per cent) but the other measured variables were essentially constant except for the pulse rate during respiration of the $O_2 + CO_2$ mixture; in the latter case the pulse rate fell slightly in 3 experiments (range −2 to −9 beats per minute, average −6).

High oxygen. In 7 experiments the test gas mixture consisted of oxygen at a partial pressure of 700 to 715 mm. Hg (corresponding to the case of a diver breathing ordinary air at a depth of around 140 ft.). Pulse rate declined in all cases; there was an average decline, compared to the control period with ordinary air, of 8 beats per minute (range −4 to −14). Also, in all cases there was a very slight rise in diastolic blood pressure, averaging 4 mm. Systolic blood pressure tended to rise very slightly and pulse pressure to fall equally slightly.

There was a small increase in ventilation (average +16 per cent, range +4 per cent to +35 per cent) and a small decrease in minute volume (average −10 per cent, range −20 per cent to +19 per cent). Cardiac "work" appeared to remain constant (average +3 per cent) while "effort" decreased somewhat (average −12 per cent). As a result the apparent "efficiency" of the heart appeared to be improved to a small extent (average +17 per cent, range −9 per cent to +145 per cent).

Discussion. Experiments on operated animals and on surviving hearts show that severe acute hypoxia tends to cause dilatation of the heart. Lorber (1942) has shown that the dilating effect in rabbits' hearts is roughly proportional to the decrease in pO_2 but that this effect only begins below a threshold level of pO_2 which is considerably lower than tolerated by the intact animal. In the experiments reported here there was no tendency to produce any dilatation of the heart in either diastole or systole. It may be suggested that the time periods were too short to allow cardiac dilatation. This is not the case; we have produced well-marked dilatation of the hearts of normal men in equally short periods by means of the drug neo-synephrine (Keys and Violante, 1942).

It is not possible to make rigid generalizations about the behavior of the blood pressure of men subjected to acute hypoxia. Inadequate control of temperature and emotional factors discredits much work on this subject. The present results were obtained under constant temperature and absence of psychic stimuli. We did not find that subjects who faint at one pO_2 show peculiar or abnormal blood pressure responses at a somewhat higher pO_2 which they can tolerate.

Schneider and Truesdell (1921) stated that syncope could be predicted by a decline in systolic and diastolic blood pressure. A similar statement, based on unconvincing data, is made by Schwarz (1936). Besserer (1936) reported that syncope could be predicted by a transient *rise* in systolic blood pressure. The present results show that syncope itself involves an abrupt fall in blood pressure and that it *may* be preceded by an abrupt rise. Syncope was frequently unheralded by any notable changes in blood pressure. Cerebral asphyxia or anemia can cause syncope and loss of the wink reflex in man within as little as 5 seconds (Kabat, Rosen and Anderson, 1942). In a state of partial anoxia it is understandable then that syncope might occur without warning. A slight shift of blood distribution could be responsible.

Gellhorn has emphasized the importance of the autonomic nervous system in asphyxia and anoxia (1941). In most, but not all, of our subjects extreme peripheral vaso-constriction occurred at the time of collapse and in one case

this was succeeded in the first minute or so of recovery by a peripheral flush fully as intense as we have ever seen produced by atropine. Hypoxia disturbs the stability of the balance between the sympathetic and parasympathetic nervous systems. The initial tendency for the sympathetic system to dominate gives way to parasympathetic dominance which is well marked and occasionally extreme in recovery. Sweating at the time of collapse is common and sometimes very marked. The skin is always moist in recovery. We have mentioned the characteristic bradycardia in recovery after collapse.

Estimation of cardiac output by the foreign-gas method requires changing pO_2 during the course of the measurement. In the hypoxic state small changes in pO_2 produce large changes in oxygenation. It is not certain that this source of error can be quantitatively eliminated; among other things the rate of equilibration with lung and cardiac tissue is unknown. Further the effective pCO_2 at the moment of oxygenation is uncertain and hence no precise calculation can be made for the relation between pO_2 and oxygen saturation. With the foreign-gas method Christensen and Forbes (1937) reported a moderate increase in minute output in hypoxia and Asmussen and Chiodi (1941) reported an average increase of 98 per cent in minute output of 3 subjects.

Ranke (1936), using the pulse wave method, reported increases in minute output in 5 subjects (8 expts.) ranging from 17 per cent to 101 per cent (average 59 per cent). A much more complete study on 10 subjects with similar methods was reported by Herbst and Manigold (1936). At altitudes of 5,000 to 8,000 meters (in a low pressure chamber) the average change in 30 measurements was +11.3 per cent in stroke volume and +41.4 per cent in minute volume. These results are quite consistent with those reported here.

Matthes and Malikiosis (1937) made estimates using a complicated method involving estimations of arterial saturation with an optical system applied to the ear and estimations of saturation of the mixed venous blood by an indirect method. The results of 3 experiments on one person indicated that minute volume is increased at altitudes of 6,000 to 7,000 meters.

All known methods for estimating minute volume in man are subject to some criticism and the method used here is no exception. However, the present method is particularly satisfactory for measurements of *changes* in stroke and minute output and it is not subject to special or systematic errors at low pO_2.

All observations reported here lead to the conclusion that the normal heart is remarkably resistant to hypoxia and that the capacity of the heart to resist this condition is not ordinarily the limiting factor for the intact normal human body. Perhaps of greatest importance is the fact that the heart may be safeguarded in hypoxia by a very marked increase in coronary flow (Wiggers, 1941).

The absence of any anginal or other cardiac complaints was notable in the many experiments in this laboratory. Persons with coronary disease, however, exhibit signs of cardiac embarrassment and suffer precordial pain under similar experimental conditions. The presence of small amounts of carbon dioxide in the inspired oxygen-deficient air has a markedly beneficial effect in most such persons (Barach and Steiner, 1941). This is in agreement with

the effects reported here. Part of the value of carbon dioxide may be in shunting away some circulation from the non-essential periphery (Gellhorn and Steck, 1938).

SUMMARY

1. The results are reported from numerous experiments in which acute hypoxia was produced in 27 normal young men. All studies were made under constant environmental conditions with the exception of the partial pressures of the gases in the inspired air. In most cases the pO_2 corresponded to that at 18,000 to 28,000 feet altitude; in some cases CO_2 at 14 to 30 mm. Hg was present. Some experiments were made with inspiration of pure oxygen. Exposure lasted from 10 to 48 minutes.

2. From roentgenkymographic measurements it is concluded that cardiac dilatation does not take place under these conditions. The stroke volume remains nearly constant in this acute hypoxia and the minute output of the heart is increased only slightly more than in proportion to the pulse rate change. Cardiac efficiency is unimpaired.

3. Carbon dioxide increases the altitude tolerance and in hypoxia with CO_2 added the pulse rate increases less than without it. Again, the heart does not dilate and the stroke volume tends to remain constant.

4. Respiration of oxygen at 4 to 5 times the normal pO_2 results in a slight decrease in cardiac "work" and "effort" with no significant change in heart size or efficiency.

5. Blood pressure responses have no certain predictive value as to whether syncope is to occur. In the present series complete syncope occurred in 10 cases. There is always a relative bradycardia in recovery from hypoxic syncope.

6. Indications were seen that acute hypoxia disturbs the stability of the autonomic nervous system.

7. It is concluded that, in normal young men, attempts to strengthen or safeguard the heart under hypoxic conditions would serve no useful purpose. The heart does not seem to be the limiting factor in tolerance to acute hypoxia.

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SEGMENTAL MOTOR INNERVATION OF THE TIBIALIS ANTERIOR AND GASTROCNEMIUS-PLANTARIS MUSCLES IN THE DOG

O. LEONARD HUDDLESTON AND CLAYTON S. WHITE

From the Department of Physiology and Pharmacology, University of Colorado School of Medicine, and Department of Physical Therapy, Colorado General Hospital, Denver

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As a preliminary to a study of the influence of stretch on the changes in skeletal muscle during degeneration and regeneration of the motor nerve supply, determinations were made of the segmental innervation of a set of antagonistic muscles of the hind limb. It was necessary to know the precise innervation of the muscles in order to establish a complete motor paralysis, and at the same time cause a minimal disturbance of the functional integrity of the animal. Accurate knowledge of the motor nerve supply was desired also for use in another phase of our problem, namely, that of producing varying degrees of partial paralysis of the muscles selected for study. The purpose of this paper is to report the segmental innervation of the anterior tibial and the gastrocnemius-plantaris complex in the dog.

METHOD. The right anterior nerve roots of L 3, L 4, L 5, L 6, L 7, S 1, S 2 and S 3 were isolated and a silk ligature tied about each motor root close to its exit from the dural canal. After sectioning the attachment of the roots to the spinal cord, a segment of 1 to 2 cm. in length was removed from the corresponding posterior nerve root. As a rule, the spinal nerve roots of the opposite side were transected. The tendon of the anterior tibial muscle was ligated and freed from its place of insertion, and those of the gastrocnemius-plantaris muscles were tied with a common ligature and detached from the tuberosity of the calcaneus. The tendinous extension of the biceps femoris which accompanies the gastrocnemius-plantaris tendons was isolated and cut. The tendons of these antagonistic muscles were attached to two calibrated isometric levers and adjusted to record simultaneously the tension myograms. Records were made of the muscular responses resulting from the stimulation of each anterior nerve root extending from L 3 to S 3 inclusive. The stimulating current was supplied by a Harvard inductorium.

RESULTS. Observations were made on a group of sixteen dogs. Satisfactory results were obtained in fourteen of the sixteen animals. Table 1 shows the qualitative, semi-quantitative and quantitative data obtained in these experiments. Examination of the data shows that the motor nerve supply of the gastrocnemius-plantaris muscles was derived from the roots of L 5, L 6, L 7 and S 1. Root L 5 was involved in only four of the sixteen animals, and only in dog 1 was it markedly involved This dog had no sacral representation. The motor nerve supply of the anterior tibial muscle was carried by L 6 and L 7 in all animals except dog 1. In this animal the nerve supply of the anterior tibial was confined to L 5. It is interesting to reiterate that this animal showed the

antagonistic muscles to be supplied by L 5, L 6 and L 7 with no sacral representation. Apparently dog 1 was an example of what Sherrington (1892) has termed a "prefixed" animal. Quantitative data obtained from measurement of the myograms are shown for the last six animals listed in table 1. The figures signify the amount of tension developed during muscular contraction and expressed in grams.

TABLE 1

Qualitative, semi-quantitative and quantitative data showing the magnitude of muscular contractions resulting from stimulation of the anterior nerve roots of lumbo-sacral nerves, L 3, L 4, L 5, L 6, L 7, S 1, S 2, and S 3

0 = not tried; − = negative response; +, ++, +++, ++++ = semi-quantitative results referring to the relative magnitude of muscular contractions; figures 411, 3526, etc. = quantitative data expressed in grams tension as revealed by the tension myograms.

DOG NO.	GASTROCNEMIUS-PLANTARIS MUSCLES								ANTERIOR TIBIAL MUSCLE							
	Lumbar segment					Sacral segment			Lumbar segment					Sacral segment		
	Root no.					Root no.			Root no.					Root no.		
	3	4	5	6	7	1	2	3	3	4	5	6	7	1	2	3
1	0	−	++	++++	+++	−	−	0	0	−	++++	−	−	−	0	0
4	0	−	0	++++	++++	0	0	0	0	−	0	++++	++	0	0	0
6	0	−	+	++	++++	+++	−	−	0	−	−	++++	++	−	−	−
7	0	0	0	++	++++	++	0	0	0	0	0	++++	++	−	0	0
8	0	−	−	++	++++	++++	0	0	0	−	0	++++	+	−	0	0
10	−	−	−	+	++++	++	−	0	−	−	−	++++	+	−	−	0
11	0	−	−	+	++++	++++	−	−	0	−	−	++++	+++	−	−	−
12	0	−	−	−	+++	++++	−	−	0	−	−	++	++++	−	−	−
21	0	0	−	+	++++	++++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	926	3466	3402	−	−	0	0	−	3174	784	−	−	−
22	0	0	−	++	++++	+++	−	−	0	0	−	++++	++	−	−	−
	0	0	−	1529	4801	4155	−	−	0	0	−	2978	1254	−	−	−
23	0	0	−	+	++++	+++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	818	4091	2627	−	−	0	0	−	2625	411	−	−	−
24	0	0	−	+	++++	+++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	538	3445	2218	−	−	0	0	−	3526	882	−	−	−
25	0	0	+	++	++++	++	−	−	0	0	−	++++	+	−	−	−
	0	0	431	1055	3230	1077	−	−	0	0	−	3095	392	−	−	−
67	0	−	+	+−	+++	++++	−	0	0	−	−	++++	++++	−	−	0
	0	−	650	875	5150	6888	−	0	0	−	−	3526	3690	−	−	0

In this group of animals the total tension developed by the anterior tibial in each instance resulted from stimulation of L6 and L7, the tension ranging from 392 to 3690 grams. The percentage tension developed by the stimulation of L 6 ranged from 48.6 per cent to 86.4 per cent, while that developed by the stimulation of L 7 ranged from 13.6 per cent to 51.4 per cent. The tension developed in the gastrocnemius-plantaris as a result of stimulating the anterior nerve roots showed a wider range of distribution. In the majority of instances the total tension was developed by the stimulation of three anterior nerve roots,

namely, L 6, L 7, and S 1, with the exception of two of the six animals in which it was necessary to stimulate four anterior nerve roots, namely, L 5, L 6, L 7 and S 1. In all animals the majority of the tension was obtained from the stimulation of L 7 and S 1, while lesser amounts were developed from the stimulation of L 6 and L 5. The tensions developed from the stimulation of L 5 ranged from 0 to 650 grams, for L 6—818 to 1529 grams, for L 7—2218 to 5150 grams, and for S 1—1077 to 6888 grams. The percentage of the total tension developed from the individual stimulation of the respective nerve roots in this group of six dogs may be summarized as follows: L 5 = 0—0—0—0—7.4 per cent—4.8 per cent; L 6 = 11.9 per cent—14.6 per cent—10.9 per cent—8.7 per cent—18.2 per cent—6.3 per cent; L 7 = 14.5 per cent—45.8 per cent—54.3 per cent—55.5 per cent—55.8 per cent—38.1 per cent; S 1 = 43.6 per cent—39.6 per cent—34.8 per cent—35.8 per cent—18.6 per cent—50.8 per cent. It is obvious that there was a variable percentage of tension produced in the gastrocnemius-plantaris muscles as a result of stimulation of the lower lumbar and upper sacral nerve roots. The individual variation and the amount of tension developed in different animals was found to be so great that it is impossible to draw any definite conclusions regarding the per cent of total tension supplied by each individual anterior root. However, it is reasonably safe to say that from 14.5 per cent to 55.5 per cent was supplied by L 7, from 18 per cent to 51 per cent by S 1, less than 20 per cent by L 6 and less than 10 per cent by L 5. The mode of percentage tension for L 7 ranged between 40 per cent and 50 per cent, and the mode for S 1 ranged between 30 per cent and 40 per cent.

CONCLUSIONS

From an investigation of the segmental motor innervation of the anterior tibial and gastrocnemius-plantaris muscles of fourteen dogs, the following facts were found:

1. The tibialis anterior muscle in the dog received its segmental motor nerve supply from L 5, L 6 or L 7. In the majority of animals two roots were concerned, L 6 and L 7, with most of the fibers from L 6. Three exceptions were noted; in one, root L 5 alone was involved, in another, most of the fibers were in L 7, while in the third, they were almost equally distributed between L6 and L 7.

2. The segmental motor nerve supply of the gastrocnemius-plantaris complex arose from roots L 5, L 6, L 7 and S 1. In nine animals three adjacent roots were involved. Eight of these received fibers from L 6, L 7 and S 1 while the other one involved L 5, L 6 and L 7. In three animals all four roots were involved (L 5, L 6, L 7 and S 1), and in two animals only two neighboring roots were concerned—L 6 and L 7 in one, and in the other L 7 and S 1.

3. As a rule most of the motor fibers of the gastrocnemius-plantaris complex were carried in two adjacent roots, namely, L 7 and S 1 This was so in ten animals. In two animals the majority of the fibers were supplied by L 6 and L 7, while in the remaining two dogs, the major portion of the fibers were carried in L 7, and the remainder of the fibers equally distributed in L 6 and S 1.

4. The experiments show that it is necessary to transect the anterior nerve

roots of L 5, L 6, L 7 and S 1 in order to insure the development of a complete paralysis of the anterior tibial and gastrocnemius-plantaris muscles of the dog.

5. As regards the ankle flexors and extensors of the hind leg, it is impossible to express a reliable percentage of total innervation that is supplied by each individual anterior nerve root.

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HUMORAL INTERMEDIATION OF NERVE CELL ACTIVATION IN THE CENTRAL NERVOUS SYSTEM[1, 2, 3]

ROBERT GESELL, E. T. HANSEN AND JOSEPH J. WORZNIAK

From the Department of Physiology, University of Michigan, Ann Arbor

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The comprehensive rôle which the acid humoral mechanism of nerve cell stimulation (Gesell, Brassfield and Hamilton, 1942) may play in the integrations of the nervous system as a whole rests on the soundness of the theory of humoral intermediation of nerve cell stimulation in the centers, a view not generally accepted. Best and Taylor (1939) believe that "Little *direct evidence* (Italics ours) can be cited in support of the concept though certain suggestive observations have been cited," and Bard (1941) believes "While there is almost universal agreement that immediate control of the slowly acting effectors by autonomic fibers is mediated by chemically specific substances liberated at the nerve terminations the evidence bearing on central synaptic transmission certainly favors the electrical theory." The reluctance of physiologists to accept central humoral intermediation on the basis of present knowledge (frequently cited references in textbooks, reviews and monographs) indicates the need of further experimentation if that concept is to play a rôle in the physiology of the central nervous system.

PROCEDURE. Dale and his associates (1929) established the humoral mechanism in simple cholinergic systems by a systematic application of elementary physiological principles: 1, the reproduction of physiological activity by acetylcholine; 2, the potentiation of indirect stimulation by an anticholinesterase; 3, the liberation of acetylcholine by indirect stimulation. We have followed the procedure of Dale and his group and added modified approaches suited to the peculiar intricacy of central nervous activity. The present discussion includes procedures 1 and 2 only.

Our observations were limited almost exclusively to the respiratory act of the dog. They were made under chloralose anesthesia, morphine urethane anesthesia and decerebration performed under evipal anesthesia.

The choice of the respiratory act possessed outstanding advantages—the abundant information on the location, structure and activity of the respiratory center, the automaticity, the even rhythm and intensity of discharge, and the ease of recording respiratory activity. Though one of the simplest of motor integrations the respiratory act possesses the main elements of completeness. Two antagonistic half-centers alternately activate and inhibit the inspiratory and expiratory muscles due to an effective mechanism of reciprocal innervation.

[1] Preliminary report: J. Worzniak and R. Gesell. This Journal Proc. **123:** 222, 1938.

[2] Preliminary report: E. T. Hansen, J. J. Worzniak and R. Gesell. Fed. Proc. **1:** 36, 1942.

[3] Supported in part by a grant from the Rockefeller Foundation.

These half-centers react delicately to chemical stimuli and to well-known sensory drives. Some of these drives run in specialized nerves and thus permit analysis of specific reflex activities in relation to the neurohumoral theory. Finally, the precise information in the activity patterns of normal breathing offers a criterion of comparison which can leave no question of whether or not duplication of normal central nervous integration has been attained by artificial administration of extrinsic acetylcholine.[4]

RESULTS. 1. *The reproduction of physiological activity by acetylcholine.* An increase in the intensity of breathing is demonstrated with several methods of administration of acetylcholine, intravenous, intra-arterial (cerebral arteries) and intraventricular onto the floor of the fourth ventricle. Injected intravenously, as in figure 1A, an augmentation of breathing occurs which in a few minutes subsides to normal. This response might well be a combined effect of acetylcholine upon the chemoceptors and the respiratory center, for Heymans et al. (1936) have demonstrated that the carotid body is highly sensitive to acetylcholine. Such a double site of action of acetylcholine is illustrated in figures 1B and 1C. In figure 1C, 0.1 mgm. of acetylcholine was injected into the right common carotid artery after denervation of the corresponding carotid body to limit its effects to the center only. In figure 1B the same amount of acetylcholine was injected into the left carotid artery with the innervation of the corresponding carotid body intact. This larger response is, therefore, regarded as the sum of peripheral and central excitation. v. Euler, Liljestrand and Zotterman (1941) "look upon the sinus region as a sort of nervous center with a peripheral localization of the same general type as in the olfactory or optical peripheral organ." Nerve cell activation is thus involved in peripheral as well as central stimulation. Our present paper however deals specifically with the controversial problem of central intermediation.

To establish a purely central action acetylcholine is injected into one of the cerebral arteries, either after denervation of all known respiratory chemoceptors or during cold block of the chemoceptor afferents (Hering's nerves and cervical vagus nerves). This procedure produces a remarkably smoothly co-ordinated hyperpnea (fig. 1D). The precaution required to obtain such results is to avoid excessive doses. If the injection is too rapid, there may be a momentary reduction of breathing similar to that in figure 1C. More often there is a perfect combination of adjustments of increased frequency of breathing, and of increased intensity of both inspiratory and expiratory components, so similar to physiological hyperpneas as to warrant the belief that a truly physiological activity has been produced by an artificial administration of acetylcholine. The shortness of the latent period following injection (about 2 sec.) points unquestionably to a central action but the ultimate proof that the response is a truly physiological activity rests on the basic similarity of the activity patterns of the opposing muscles to those of normal breathing. This point will be established below.

[4] We wish to thank Merck and Company, Inc., for the acetylcholine used in these researches.

2. *On the impossibility of segregating the two dominating central excitatory effects of acetylcholine.* From the observations of others on simple cholinergic systems,

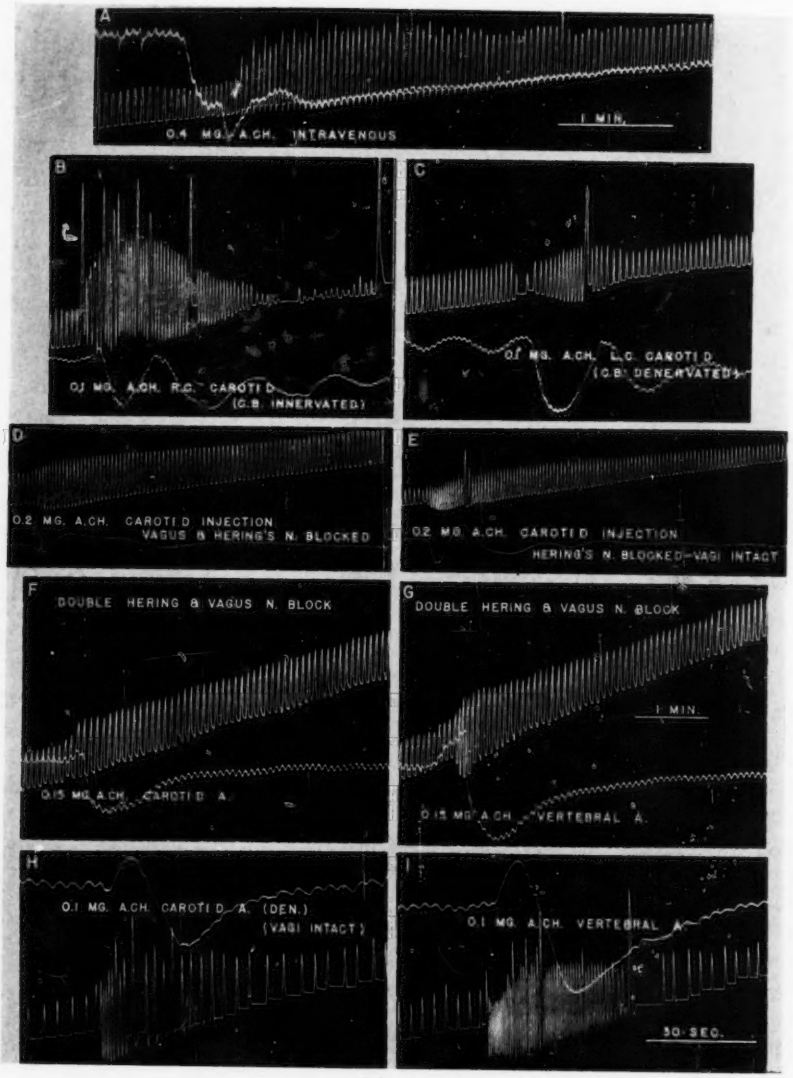


Fig. 1

acetylcholine should exercise two central excitatory effects: 1, a direct excitation at the synapses of the respiratory nerve cell; 2, a potentiation of the effects of all impulses impinging at these nerve cells. If that assumption holds, acetyl-

choline would be expected to produce a varied response of the centers consistent with the general volume and pattern of impinging signals existing at the moment. A comparison of figures 1D and 1E seems to support this view for the experimental conditions differ presumably only in the flow of sensory signals.[5] In 1D all vagal afferent impulses are eliminated by double vagal block whereas in 1E they are returned by vagal deblocking before acetylcholine is injected into the carotid artery. In the latter the frequency of breathing is increased much more and the amplitude is increased much less. Since acetylcholine could hardly have reached the aortic bodies in time or in concentrations to account for the altered response, central potentiation of the highly excitatory vagal proprioceptive reflexes seems to be the likely explanation of the difference of response. This explanation suggests the impossibility of segregating the direct excitatory action of extrinsic acetylcholine from the potentiating action on "intrinsic acetylcholine" (i.e., acetylcholine deposited by nerve impulses). We believe that these two effects actually do work hand in hand in the central nervous systems, under all conditions, experimental or otherwise. The proof of this joint action, through the so-called potentiation of impinging nerve impulses will also be presented below.

3. *The factor of mass stimulation.* Figures 1F and 1G show quantitative differences in the central effects of equal amounts of acetylcholine when injected into the carotid and vertebral arteries respectively. Double Hering nerve and double vagal block eliminate the effects of peripheral chemoceptor stimulation. The larger response (fig. 1G) occurred when acetylcholine was injected into the vertebral artery indicating that acetylcholine injected into the vertebral artery either reaches more nerve cells or reaches them in greater concentration.

If the same comparison of carotid and vertebral injections is made again after re-establishing the pulmonary proprioceptive reflexes by deblocking the vagus nerves (figs. 1H and I), vertebral injections show once more a similar if not greater superiority of stimulation than carotid injections. The much greater hyperpnea produced by vertebral injection could, therefore, be interpreted as the sum of a greater direct stimulation of the center plus a greater potentiation of a greater volume of vagal excitatory proprioceptive signals.

4. *Fundamental implications raised by the central effects of extrinsic acetylcholine.* How can a co-ordinated response to a shapeless stimulus such as arterial injection of acetylcholine be explained? Surely this stimulation must be devoid of such orderly or temporal arrangements emphasized by the electrical theory of transmission. Admitting that sensory patterns and temporal arrangements are wanting, that acetylcholine injected into the vertebral artery reaches the respiratory center and stimulates each and every synapse, and that all of these myriads of stimulations are occurring in a perfectly disorderly and asynchronous manner, what substitute mechanism of nervous integration is there to offer? Activation of heterogeneous synapses would impose a simultaneous excitatory influence upon both half-centers, yet only one-half center discharges at one

[5] We have been unable to relate changes in breathing to blood pressure fluctuations associated with injections of acetylcholine.

time. Simple reciprocating interconnections might provide the mechanism needed to direct this drive. Just as the steam valve in the locomotive shunts the steam pressure (sum total of asynchronous impacts) from one piston to another so is reciprocal inhibition conceived to be the nervous gadget which shifts the relatively steady head of nervous drives (sum total of asynchronously impinging impulses) from one half-center to another and thus converts a chaotic stimulation into an orderly event (Gesell, 1940).

We believe experimental support for this concept of nervous integration is found in the effects of sensory nerve stimulations on breathing, in which either the inspiratory or the expiratory components of dual excitatory afferents (of the vagus and the saphenous and of the chemoceptors) add one to another as one readily adds the stimulating effects of one injection of acetylcholine to another (Gesell and Hamilton, 1941). During the expiratory phase of breathing when the expiratory half-center dominates the respiratory act, one expiratory drive adds to the other, regardless of its origin or of the temporal relation of one group of signals to the other (faradic stimulation from individual coils was used). During the inspiratory phase of breathing, heterogeneous inspiratory excitatory impulses add one to another in a similar way. Consequently it seems that quantity of impulses or more specifically, the summation of depositions of lingering acetylcholine into a smoothed effect becomes the critical factor in reflex excitation just as the amount of extraneous acetylcholine injected is the factor determining the intensity of response. Is it not advisable to look for neuro-architectural machinery in which the pattern of *structure* rather than pattern of *impulses* exercises the dominating influence on motor integration?

The first prerequisite in this concept is the proof that the respiratory center is capable of responding in a rhythmical way to a steady drive. That was provided many years ago by the continuance of rhythmical breathing after dorsal root deafferentation of the respiratory center (Marckwald, 1888) and more recently by the continuance of rhythmical discharges in the phrenic nerve after immobilization by curari (Winterstein, 1911; Bronk and Ferguson, 1935).

The second prerequisite is the proof that the discharge of the respiratory center robbed of its normal periodic afferent drive still has the same general basic pattern of activity of a physiologically intact animal. Here, too, the answer is positive (Gesell, Atkinson and Brown, 1940) for action potential studies have demonstrated that the activity of the respiratory center during curari poisoning is not only rhythmical but is organized in co-ordinated details. It must follow that steady drives which motivate breathing, regardless of their source (chemoceptors, nociceptors, proprioceptors, etc.) are converted by the inherent structure of the respiratory center into geometric patterns of motor activity characteristic of normal breathing. This pertinently conforms with the requirements of neurohumoral integration, for a relatively slow rate of destruction of acetylcholine is adaptable for a fusion of incoming impulses into a common steady drive. The actual "pooling" of acetylcholine, therefore, becomes an integral part of nervous integration.

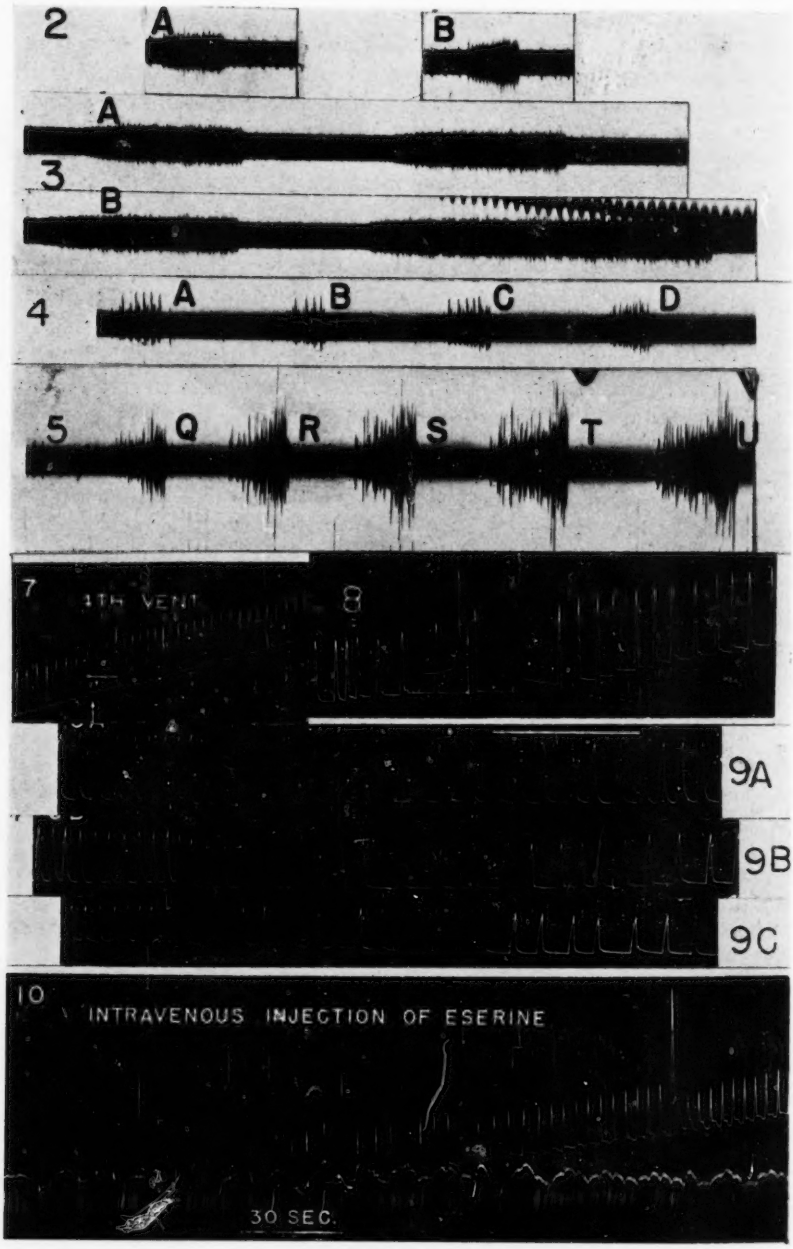
The third step in the concept of structural dominance in motor integration

consists in determining the basic activity pattern of the response of the respiratory center to the shapeless stimulation provided by arterial injection of extrinsic acetylcholine. Specifically—are the geometrical activity patterns of normal breathing retained or are they lost in acetylcholine hyperpnea? The answer to this question is found in part in figures 2, 3, 4, 5 and 6.

5. *Proof of the basic similarity of physiological hyperactivity of the respiratory center and that produced by extrinsic acetylcholine.* The slowly augmenting inspiratory pattern of eupnea is not only preserved but actually intensified by acetylcholine hyperpnea. (Compare fig. 2B with 2A, 3B with 3A and 5Q, R, S, T and U with 4A, B, C and D. The fusillades in figs. 2 and 3 are recorded from the phrenic nerve and those in figs. 4 and 5 from respiratory muscles.) It must be recalled that the characteristic triangular configuration of the normal eupneic inspiratory pattern shows a progressively increasing activity during the course of each individual inspiration and that the increasing amplitude of the electrical record is the combined result of an increasing frequency of activity (either of nerve cells or muscle fibers) and of a progressive recruitment of newly activated units (Gesell, Atkinson and Brown, 1941). Both factors lead to a growing summation of increasingly coinciding potentials which reaches a maximum at the end of the inspiratory act.

The accentuation of the inspiratory activity pattern (fusillade) by arterial injection of acetylcholine is evidence in itself that the standard integration of normal inspiratory activity is employed by the respiratory center in extrinsic humoral hyperpnea. Note the increasing fusillades in figures 5Q, R, S, T and U and compare with the eupneic fusillades in figures 4A, B, C and D. But if the electrodes are fortunately situated in a relatively inactive portion of a muscle, they will register the individual activity of one or more newly recruited units and reveal step by step the manner in which eupnea is intensified and the characteristic activity pattern retained during hyperpnea produced by the central action of acetylcholine (see figs. 6A to M).

When breathing is relatively stable, as it commonly is after vagotomy, the performance of individual muscle units is also relatively dependable, to the extent that an incompletely recruited muscle unit will begin to twitch at an appointed moment and at an appointed increasing twitch frequency schedule, and that it will deliver a relatively uniform number of twitches per inspiration, let us say 7 as in figure 6A. If physiological hyperpnea were now to be produced that particular unit would immediately contribute to the respiratory response in several ways. It would begin to twitch longer and at a higher frequency and it would deliver a greater number of twitches per inspiration. While this unit was progressively reaching its maximum activity other newly recruited units would join the contraction at the very end of inspiration and go through a similar process. The same general scheme of integration holds for hyperpneas produced by the central action of acetylcholine. This is illustrated in figures 6A, B, C and D where 0.12 mgm. of acetylcholine is injected into the carotid artery of a chemoceptively denervated dog. The effects come on in less than two seconds and therefore are unquestionably central. The electrical changes of the internal



Figs. 2, 3, 4, 5, 7, 8, 9, 10

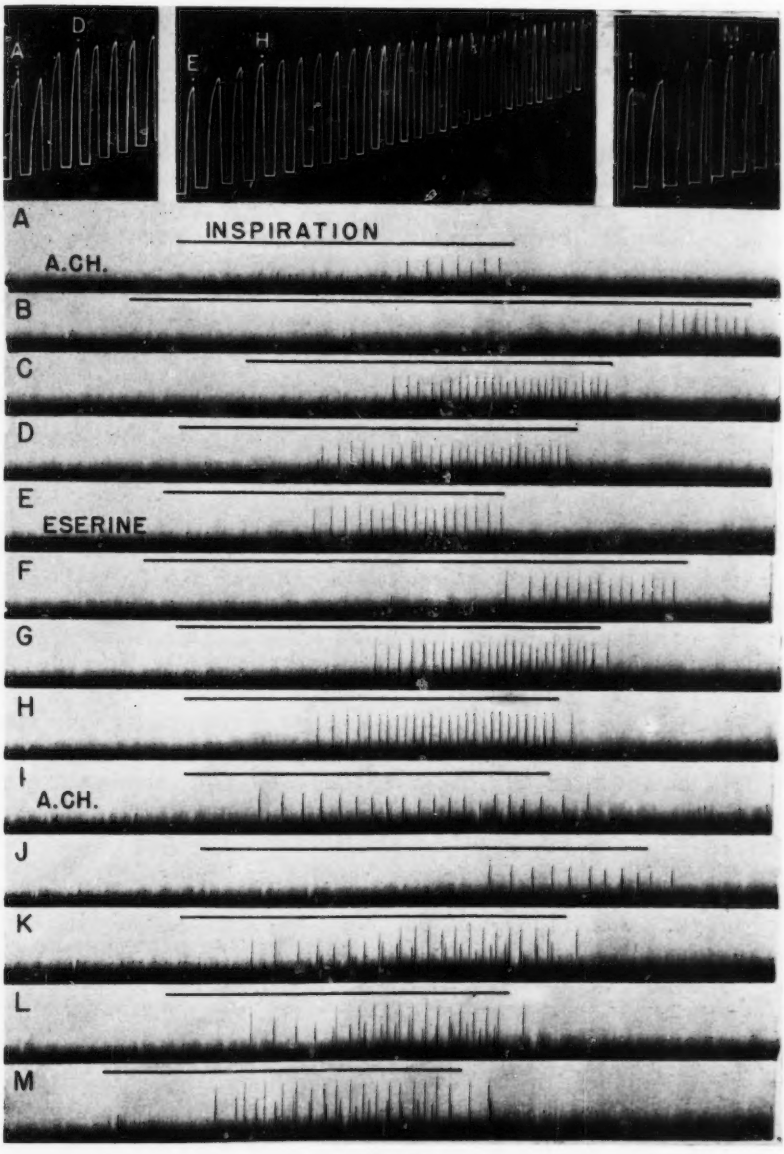


Fig. 6

intercostal muscle were recorded on a rapidly moving paper to give better definition to the potentials. The smoked record which runs much more slowly is, however, suited to show the changes in depth of breathing and is included in the

figures. The discrete potentials superimpose themselves upon a background fusillade of slowly developing intensity (very faintly visible). The appearance of the discrete twitches in the latter third of normal inspiration 6A is evidence that the normal mechanism of recruitment of muscle units is at work to meet the need of increasing mechanical energy of that individual inspiration, and respirations 6B, C and D, appearing immediately after acetylcholine show the extension of the normal mechanism of recruitment into hyperpnea. The incompletely recruited unit still speeds its pace with each inspiration in hyperpnea as it did in eupnea. It twitches for a longer period and at a higher maximum frequency and in respiration 6B it delivers 10 tugs instead of 7. Often the first inspiration of hyperpnea develops very slowly and by its prolongation tends to throw the recruited unit off its regular schedule when judged by the moment of onset of twitching, but that disturbance disappears in the next inspiration, 6C. Here the activity begins much earlier in the inspiratory phase, it lasts twice as long, and delivers 2.6 times as many twitches, which means an appreciable increase of gross frequency of twitch. The maximum frequency of twitch is higher than it is in 6B, and approximately twice that of the control inspiration 6A.

Figures 6E, F, G, and H show that the same mechanism of gradation of hyperpnea is operative when eserine (0.4 mgm.) instead of acetylcholine is injected into the carotid artery. The prolonged and slow development of the first inspiration after eserine (6F) disturbs the schedule as it did above, but in the following inspirations this unit responds in a manner typical for acetylcholine. It twitches over a longer portion of the inspiratory phase, and at a higher maximum twitch frequency and it delivers approximately 27 tugs as compared with the initial 17.

In figures 6I, J, K, L and M there are two units whose activity can be followed. There is a completely recruited unit which during eupnea (fig. 6I) contracts from the very beginning of inspiration and through the entire inspiratory phase. There is also an incompletely recruited unit which becomes active in the second breath after acetylcholine, 6K. This unit can be distinguished from the completely recruited unit by its taller electrical deflection. As is usual for this animal, the activity schedule of the muscle units is disturbed in the first prolonged inspiration after injection. The total number of twitches delivered by the completely recruited unit falls from 20 to 11, but in the next breath which is of the same duration as that of eupnea, twitching is back to schedule (20 per inspiration). That agrees with our concept that a completely recruited unit cannot be pushed by increasing stimulation beyond a set limit, which in turn agrees with the decrease of the number of twitches of this unit as the duration of the succeeding inspiration shortens. The incompletely recruited unit however answers to the increasing respiratory drives in the usual way. In breath 6K it begins to twitch only after its fully recruited mate had delivered its 7th twitch, in breath 6L it becomes active after the 5th twitch, and in breath 6M it has almost reached complete recruitment. It now joins the contraction after the second twitch of its mate. In breath 6K it contributes 14 tugs, in breath 6L which is considerably shorter it nevertheless delivers the same number of twitches which is indicative of increased activity. In breath 6M, which is also short, it delivers a total of 18

twitches. It is apparent that these two muscle units have followed the orderly rules of integration which are expected in physiological hyperpnea.

We have the evidence that expiratory muscular activity is intensified by acetylcholine and eserine in the same manner as is the activity of the inspiratory muscles. The triangularis sterni which contracts in the slowly augmenting way, characteristic of all inspiratory muscles, shows an intensification of activity and of pattern like that illustrated in figures 5Q, R, S, T and U, and 6A to M. It remains only to state that we have also abundant electrical data showing a highly co-ordinated intensified interaction of the half-centers in which the frequency of alternation of activity is greatly augmented in conjunction with augmented intensity of discharge of the individual half-centers. The electrical records are the counterpart of what might be expected in figure 1H where the spirometer tracing clearly indicates greater power and greater frequency of inspiratory and expiratory contractions.

It is, therefore, suggested that the electrical data on the respiratory muscles contain the direct evidence required to establish the duplication of central co-ordinated nervous integration common to physiological hyperpnea by extrinsic acetylcholine.

6. *Some effects of acetylcholine placed on the floor of the fourth ventricle.* Sensitivity of the fourth ventricle to chemicals (Nicholson, 1936; Nicholson and Sobin, 1938) recommends localized application of minute quantities of acetylcholine and eserine as employed by Miller on the cortex. Simple hyperpnea is the common effect (fig. 7). Sometimes breathing is intensified but retarded (fig. 8) as it is by intravenous injection of eserine (fig. 10). Both inspiration and expiration are strengthened, the latter holding inspiration in abeyance and accounting for the prolonged expiratory phases. These results suggest a central potentiation of the dual excitatory vagal proprioceptive reflexes for the vagi are intact. The breathing resembles that resulting from sustained inflation of the lungs which is known to increase the dual excitatory drive of the vagus nerve (Gesell, Brookhart and Steffensen, 1937; Gesell, 1940). These analogous effects indicate that intensification of stretch receptor activity by pulmonary inflation and potentiation of normal unintensified stretch receptor reflexes by extrinsic acetylcholine are equivalent phenomena.

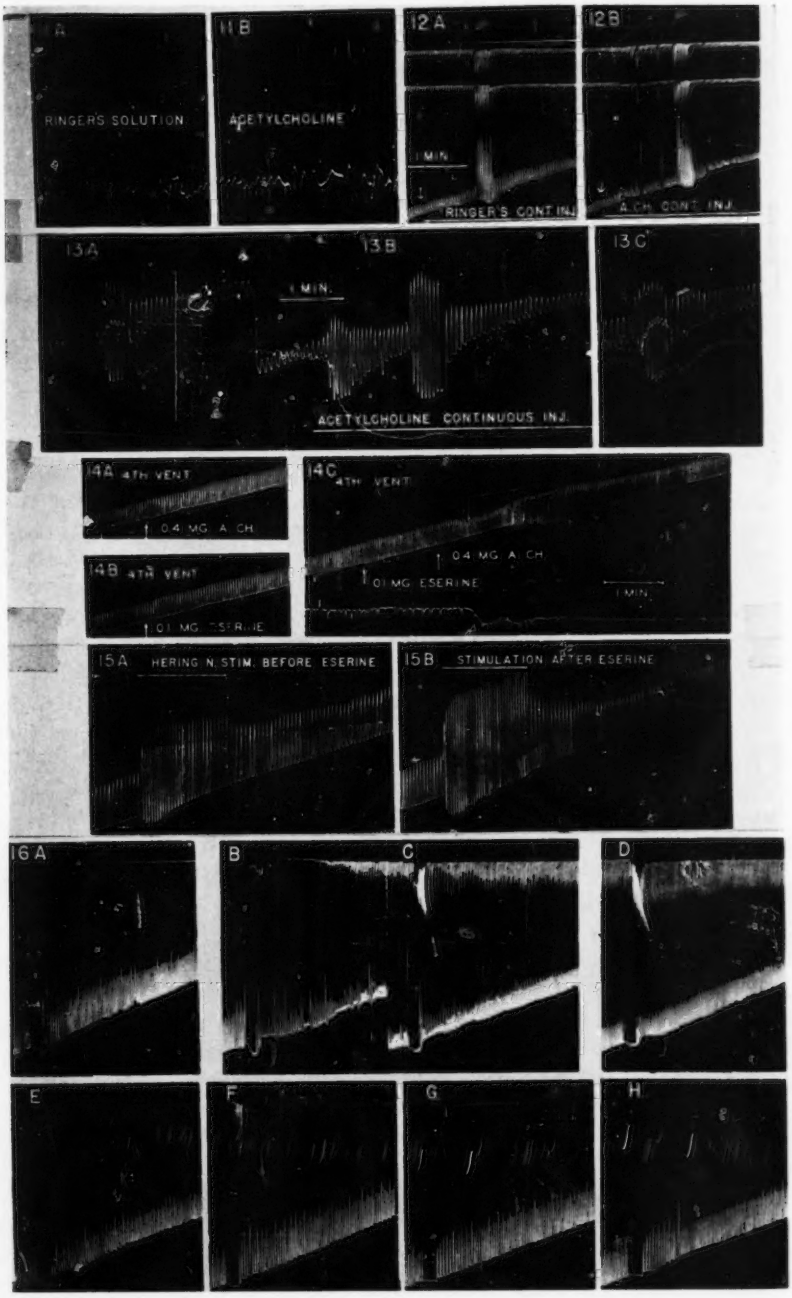
In figure 9A where the vagi are cut, the powerful inspiratory excitatory reflexes of the vagi are missing. The effects are predominantly expiratory.

7. *The similarity of effects of extrinsic and intrinsic acetylcholine.* Sporadic deep breaths and high frequency of breathing (figs. 1A, B, H and I and 12B) are rather characteristic effects of extrinsic acetylcholine in vagally intact animals whereas a slower and more even type of breathing in which inspirations are markedly prolonged is more peculiar to the effects of acetylcholine in vagotomized individuals (see figs. 1D and F). The respiratory response to chemical stimulation of the carotid body or to faradic stimulation of Hering's nerve (both of which in the light of the evidence of this paper releases intrinsic acetylcholine) is modified in the same way by the elimination of the vagal reflexes (Gesell, 1940). Note the prolongation of second inspiration in figure 3B by acetylcholine. This indicates

that the administration of extrinsic acetylcholine is comparable in its effects with those of a continuous release of intrinsic acetylcholine by faradic stimulation of Hering's nerve. We believe that either extrinsic or intrinsic acetylcholine is capable of providing comparable steady states of stimulation and that both potentiate and consequently interact in similar ways with incoming impulses. Thus, if powerfully stimulated inspirations (Hering nerve stimulation or extrinsic acetylcholine) are unsupported by the inspiratory excitatory vagal reflex and its accompanying inspiratory interrupting component (expiratory excitatory drive) inspirations tend to be deep but slow (compare figs. 1D and 1E, 1G and 1I, and 1F and 1H). If on the contrary the inspiratory excitatory reflex and its interrupting component are intact, inspirations tend to be swift and brief and of uneven depth (see figs. 1B, H and I).

8. *The central potentiation of reflexes by acetylcholine.* Dale and his associates have regarded potentiation of indirect stimulation or of extrinsic acetylcholine by eserine or prostigmine as an important step in the proof of humoral intermediation in all outlying cholinergic systems (including the sympathetic and parasympathetic ganglia). Various attempts made from time to time to establish potentiation in central humoral intermediation have met with less convincing results than those on the simpler systems where potentiation is now accepted as fact. The uncertainty rests on the variability of findings. A single reflex is found to be either strengthened or weakened, or one particular reflex is found to be intensified while another is paralyzed. This is hardly surprising or contradictory if it be remembered that the nerve cells of the central nervous system are continuously bombarded by impulses of varying and unknown number, that acetylcholine is capable of producing diametrically opposite effects (stimulation or paralysis depending upon its concentrations), and that inhibition of one reflex may be the expression of excitation of another reflex entirely overlooked.

The observation that acetylcholine produces rapid and forceful breathing when supported by the highly excitatory vagal proprioceptive reflexes (figs. 1H and I) was interpreted above as a possible example of potentiation. More direct evidence for this important phenomenon was sought with artificially induced reflexes. Figures 11A and 11B show the effects of 1.5 mgm. of acetylcholine in Ringer's solution placed on the floor of the fourth ventricle upon the respiratory response to pulmonary inflation. The lungs were inflated by weighting a specially constructed steel spirometer, floating on mercury (Gesell and Moyer, 1935), which allows a continuous recording of breathing. The retardation of breathing during pulmonary inflation attended by marked intensification of the inspiratory act and prolongation of the expiratory phase (11A and 11B) is evidence of an active stimulation of the vagal proprioceptive reflexes. Plain Ringer's solution before inflation of the lungs had no effects upon breathing and, therefore, presumably none during inflation. Acetylcholine in Ringer's solution, however, called forth a preliminary retardation of breathing of its own and a prolonged compression of the lungs (witnessed by the downward stroke of the spirometer tracing) and a sharp increase in the inspiratory excursions. This retardation of breathing before pulmonary inflation is interpreted as a dominant expiratory excitation holding inspiration in abeyance through reciprocal in-


A
RINGER'S SOLUTION
11 B
ACETYLCHOLINE
12 A
1 MIN.
RINGER'S CONT. INJ.
12 B
A. CH. CONT. INJ.
13 A
13 B
1 MIN.
ACETYLCHOLINE CONTINUOUS INJ.
13 C
14 A 4TH VENT
0.4 MG. A. CH.
14 B 4TH VENT
0.1 MG. ESERINE
14 C 4TH VENT
0.4 MG. A. CH.
0.1 MG. ESERINE
1 MIN.
15 A HERING N. STIM. BEFORE ESERINE
15 B STIMULATION AFTER ESERINE
16 A
B
C
D
E
F
G
H

Figs. 11–16

hibition. The greater retardation of breathing during increased pulmonary inflation is regarded as a potentiation of the dominating expiratory reflex.

Figures 12A and B show potentiation of hyperpnea produced by faradic stimulation of right Hering's nerve. Chemical stimulation of the carotid bodies was avoided by appropriate crushing of both Hering's nerves. The vagus nerves were left intact. Acetylcholine evoked an increase of hyperpnea of approximately 35 per cent (10 instead of 7 deep breaths). Much greater potentiation is not uncommon. The heightened rhythm of the breathing is interpreted as a potentiation of the vagal proprioceptive reflexes which are normally so active in driving both half centers and whipping up the frequency of breathing.

In figures 13A, B and C both vagus nerves as well as both Hering's nerves were cut. Hering's nerve was then stimulated for periods of 30 seconds before, during, and after injection of acetylcholine into the vertebral artery. In contrast to figure 12 in which stimulation of Hering's nerve had vagal proprioceptive support the effects of potentiation were on the amplitude rather than on the frequency of breathing. This increase in amplitude was primarily inspiratory.

To sum up: Figure 11 illustrates potentiation by extrinsic acetylcholine of an expiratory reflex, figure 13 a disproportionate potentiation of an inspiratory reflex, and figure 12 a relatively even potentiation of inspiratory and expiratory activity because the frequency of breathing is increased.

9. *Central potentiation of the respiratory response to extrinsic acetylcholine by eserine.* Figures 14A, B and C show the potentiating effects which eserine (placed on the floor of the fourth ventricle) had on the respiratory response to similar placement of 0.4 mgm. of acetylcholine. Before eserine (14A) acetylcholine had no apparent effects. After two placements of 0.1 mgm. of eserine (14B and C) which by themselves had no effects, 0.4 mgm. of acetylcholine produced a definite increase of breathing (14C).

Figure 9B shows a potentiation by eserine of a predominantly expiratory effect produced by acetylcholine on the floor of the fourth ventricle after double vagotomy. Compare with the effects of acetylcholine before eserine in figure 9A.

10. *Central potentiation of artificial reflexogenic stimulation by eserine.* Normally faradic stimulation of the chemoceptor afferents is expected to increase the depth of both inspiration and expiration illustrated in figure 15A (Gesell, 1940). This probably depends upon the simultaneous impingement of chemoceptor impulses at both the inspiratory and expiratory half-centers. Deeper inspirations and deeper expirations produced by stimulation of Hering's nerve after eserinization (fig. 15B) point to a potentiation of each of the dual excitatory effects of the chemoceptor impulses, i.e., to a greater accumulation of intrinsic acetylcholine at the individual half-centers.

Figure 16A to H shows the effects of stimulation of the superior laryngeal nerve before and after injecting 1 mgm. of eserine (stimulation at intervals of about 1, 3, 8, 18, 23, 42, and 58 min. after injection). The usual expiratory compression of the lungs (see spirometer tracing) agrees with the predominantly expiratory excitatory effect of this nerve. This compression was increased in succeeding stimulations B, C, D and E, for a period of 23 minutes following eserinization. Following record F potentiation of this reflex disappears.

The response of the facial muscles to stimulation of the superior laryngeal nerve shows even more striking potentiation (downstroke inspiration). During eupnea (fig. 16A) the response is weak and subsides quickly. One minute after eserine (fig. 16B) the response is slightly greater, and in the next ten stimulations there is a rapid, powerful, rhythmical response followed by prolonged after-potentiation. These effects decrease slowly and vanish within one hour after the injection of eserine. The prolonged "after-hyperpnea" in the facial muscles following stimulation is interpreted as an "after-discharge" caused by excessive pooling of highly protected acetylcholine.

The appearance of accessory respiratory contractions of the facial muscles in record B is most probably an expression of a slowly developing potentiation of the physiological reflexogenic drives of these muscles resulting from the prolonged effects of eserine.

Discussion. The reproduction of physiological activity in the respiratory center through the intermediation of extrinsic acetylcholine is significant in several ways. First of all it differs from the reproduction of activity in the outlying cholinergic systems (nerve muscle units, e.g.) in its composite nature, i.e., in the interaction of interrelating parts (true nervous integration). Contrast the peripheral with the central. A motor nerve impulse releases acetylcholine at the motor end plate and produces a single twitch in the corresponding muscle unit. Such twitches are reproducible by close and rapid arterial injections of a minute quantity of acetylcholine. Tetanic contractions can also be reproduced either by a massive or a prolonged administration of acetylcholine. So long as threshold concentrations of acetylcholine exist, the muscle will respond repeatedly to this steady stimulus. Such reproductions of activity are but examples of physiological stimulation in its simplest forms and are scarcely to be regarded as demonstrations of nervous integrations.

On the other hand when extrinsic acetylcholine acts upon the respiratory center a composite of highly integrated changes in nervous activities develops. 1. An increased frequency of alternation of inspiration with expiration. 2. An increased depth of inspiration. 3. An increased depth of expiration. 4. Earlier interruption of inspiration. 5. Earlier interruption of expiration. 6. A more rapid acceleration of inspiratory muscle twitching during the course of inspiration. 7. A more rapid and greater recruitment of active inspiratory units. 8. A more rapid acceleration of expiratory muscle twitching during expiration. 9. A more rapid and greater recruitment of active expiratory units during expiration. 10. A most probable increased intensity of reciprocal inhibition between half-centers for the increased intensity of the forces of stimulation at both half-centers must be held in reciprocal check.

All of these changes are the result of an exceedingly crude stimulation which has but two attributes—quantity and duration. Thus neuro-architectural structure was concluded to be of paramount importance in breathing. The question of the relative importance of architectural and sensory pattern is frequently raised and notably by Weiss (1941), who has shown that transplanted sections of the spinal cord exhibit rhythmical activity which is augmented by increased activity of the host, suggesting a similarity to experimental hyperpnea produced by extrinsic acetylcholine.

While the volume and the receptor source of afferent impulses are of undoubted value in motor integration it is nevertheless pertinent to realize that the inherent architectural machinery of the respiratory center and its inherent activity can and does actually determine the pattern of inflowing signals (Gesell, 1940). For example, the progressively augmenting activity of the inspiratory center during each inspiration stretches the lungs, increases the discharges of the stretch receptors, and augments the inspiratory excitatory component of the stretch reflex thereby intensifying the inherent inspiratory activity which is in progress. Inherent central activity and centrally motivated proprioceptive reflexes are consequently of the same progressively augmenting pattern and therefore build smoothly one into the other. Thus vicious cycles must develop which require co-ordinated machinery to interrupt the pyramiding activity. Logically one looks to simultaneous pyramiding stimulation at the opposing half-center to accomplish this end.

The factor of mass stimulation is in favor of the predominating rôle of neuro-architecture for in the greater response of the respiratory center to greater quantities of acetylcholine the typical pattern of nervous activity is retained or even intensified.

All findings seem to fit the view that acetylcholine potentiates the effects of acetylcholine. This potentiation may be merely the result of a simple addition of acetylcholine at the site of stimulation. In order that this phenomenon of summation (accumulation) may play a rôle in nervous integration it is essential that a fraction of the acetylcholine released by a nerve impulse should outlive the interval between impinging signals. The greater the sum total of impinging impulses the greater should be the accumulation of acetylcholine. Consequently the myriads of impulses composing the inspiratory excitatory reflex should increase in power by virtue of the pyramiding effects of increasingly large residual fractions of acetylcholine accumulating at increasing frequencies as the rate of discharge of the stretch receptors mounts. Thus greater power is very simply provided as it is needed.

Pooling of acetylcholine at the cardinal points in any nervous circuit becomes the paramount tool of nervous activity. Control of pooling becomes the means of gradation, lack of control the source of inco-ordination. Robbed of its normal supply of impulses a nervous circuit must labor under disadvantage.

Granted that humoral intermediation of nervous integration has been demonstrated in the respiratory act it seems fair to assume that a similar mechanism functions in *all* nervous integrations. On that point we extend the logic of Forbes (1939) who stated "if it is finally established that intercellular transmission is chemically mediated in such widely different systems as neuro-muscular junctions of smooth and striated muscle and the synapses of ganglia, the operation of a wholly different mechanism in the histologically similar synapses of the central grey matter would be a most surprising anomaly".

SUMMARY AND CONCLUSIONS

Central neuro-humoral nerve cell activation was studied on the respiratory act of the dog.

It was found that acetylcholine produced hyperactivity of the respiratory

center whether injected intra-arterially or applied to the floor of the fourth ventricle.

This effect was in relation to the concentration of acetylcholine at the center as revealed by the graded response to graded injection and by the greater effect of intravertebral than intracarotid injections after denervation of the carotid and aortic chemoceptors.

The activity produced was essentially a normal hyperpnea showing the characteristic series of changing and co-ordinated events (about ten in all) during a respiratory cycle.

The response to acetylcholine was modified by the concurrent balance of incoming impulses as shown by the difference with intact and blocked vagi.

Acetylcholine injections (extrinsic acetylcholine) had an additive effect to that liberated by incoming impulses (intrinsic acetylcholine) as shown by combining either Hering nerve stimulation or the lung inflation reflex with acetylcholine injections.

Eserine potentiated the action of acetylcholine (extrinsic and intrinsic) as shown by the increased stimulation of acetylcholine deposited on the floor of the fourth ventricle and the greater and more prolonged reflexogenic response to faradic stimulation of the superior laryngeal and Hering's nerve. Potentiation of activity was demonstrated in both half-centers.

Because the crude and shapeless chemical stimulation produced by central injection of acetylcholine evoked a highly co-ordinated activity it was concluded that neuro-architectural patterns rather than sensory patterns of impinging impulses exercise the dominant rôle in nervous integration.

It was proposed that the basic conclusions reached in these studies on the respiratory act are applicable to the central nervous system in general.

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THE EFFECT OF PANCREATECTOMY ON FAT ABSORPTION FROM THE INTESTINES[1]

CORNELIUS VERMEULEN, FREDERICK M. OWENS, JR. AND LESTER R. DRAGSTEDT

From the Department of Surgery of the University of Chicago

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The recent development by Whipple and his associates (1) and by Brunschwig (2) of methods for the radical removal of carcinoma of the ampulla of Vater and of the pancreas has emphasized anew some old problems concerning the relation of this organ to the digestion and absorption of fat in the intestines. It is now well established that the exclusion of pancreatic juice from the intestines by pancreatectomy or by successful occlusion of the pancreatic ducts produces a marked impairment in the absorption of fat and proteins. The early observations of Pratt and his associates (3) have been confirmed by many workers in this field. Coffey, Mann and Bollman (4) studied the fecal excretion of fat in normal, pancreatectomized dogs, and animals in which the pancreatic juice had been excluded from the intestines by ligation of the pancreatic ducts or by external pancreatic fistula. In the normal animal only 2 to 4 per cent of the ingested fat was recovered in the feces, whereas in the other preparations from 54 to 76 per cent of the fat fed was not absorbed. Confirming the observations made by many others these workers also reported that from one-third to one-half of the unabsorbed fat appeared in the feces in the form of free fatty acids. According to Nothmann and Wendt (5), however, this splitting of the fat occurs chiefly in the large intestine presumably through bacterial action and the fat in the upper small intestine is largely unhydrolyzed. When a fatty acid such as oleic acid was fed to depancreatized dogs, normal absorpton took place, i.e., only 13 to 34 per cent appeared in the feces. If confirmed, these observations would account for the defect in fat absorption after pancreatectomy exclusively on the basis of failure of digestion of the fat of the food by the pancreatic lipase in the duodenun and jejunum.

In the present investigation, a study was made of the absorption of a neutral fat (olive oil) and its corresponding fatty acid (oleic acid) in pancreatectomized dogs. Normal dogs were used as controls and only such pancreatectomized animals as had completely recovered from the operation and were in otherwise good health were used in the experiment. Two methods were used to study absorption. In the first method, analysis of the blood for total lipids was done at intervals after the administration of the test fat. In the normal animal a characteristic lipemic curve is known to occur following fat feeding. We, therefore, investigated the effect of administration of both olive oil and oleic

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acid on the blood lipids in normal control and in pancreatectomized dogs. In a second experiment, the amount of fat absorbed was determined by a study of the fat in the feces after the feeding of a measured amount of olive oil or oleic acid.

Absence of alimentary hyperlipemia in pancreatectomized dogs. Healthy dogs weighing from 7 to 12 kgm. were operated upon and the entire pancreas removed. Postoperatively insulin was given to all dogs twice daily. The insulin dosages were adjusted to limit glucose excretion to 4 to 6 grams in 24 hours. Fat absorption tests were not begun until the dogs had recovered from the operation and were eating well. Food was then withheld for a period of 18 to 48 hours during which time no insulin was given. As a control for subsequent studies the blood lipids for the 12 hours following the fasting period were determined at 3 hour intervals on four dogs which were given no fat. The blood lipids in these dogs showed no striking change throughout the twelve hour period and satisfied us that if no fat is given the blood lipids remain relatively constant in pancreatectomized animals in the fasting state. The effect of administration of fat upon the blood lipids was then studied. In the first experiments 50 cc. of olive oil were given by stomach tube, but the administration of oleic acid by stomach tube caused most of the dogs to vomit within a short time. Therefore, other means of introducing the fat were sought. Under anesthesia (ether or nembutal), with the abdomen open, the fat was injected directly into the duodenum in repeated small quantities with a needle and syringe. But even in normal animals lipemia did not occur. We believed that this was an effect of the anesthesia. Accordingly a method was devised to enable direct injection of the fat into the duodenum in the absence of anesthesia. This was done by inserting a de Pessar catheter into the duodenum, bringing the end of the catheter out through the abdominal wall. After complete recovery from the operation the dogs were then fasted. On the morning of the test oleic acid was injected through the catheter in small quantities until 50 cc. had been given over a period of an hour.

Blood was drawn from the femoral vein at the time of fat administration and at frequent intervals thereafter for eight to twelve hours. Serum lipids were determined by a modification of Bloor's method, previously described from this laboratory (6). The results obtained are summarized in figure 1. The initial or control serum lipid is plotted at the zerobase line and subsequent determinations appear as deviations above or below this level. The solid line curve for normal dogs represents the average obtained in two animals and shows that the blood lipid rises to a maximum of 330 mgm. per cent three hours after the oral administration of 50 cc. of olive oil and then gradually declines. The solid line curve for pancreatectomized dogs represents the average of at least one experiment on each of eighteen animals. In no instance did hyperlipemia occur. The dotted line curve summarizes experiments on four depancreatized dogs and shows that the administration of pancreatic juice does not restore the normal hyperlipemia due to olive oil feeding. These animals were given 200 cc. of fresh active pancreatic juice by stomach tube twice daily for seven days and

then on the morning of the absorption test 200 cc. of juice was given along with the olive oil. The broken line curve represents the average of one experiment on each of six depancreatized dogs and shows that raw pancreas is equally without effect in restoring the normal hyperlipemia in response to olive oil feeding. One hundred grams of fresh raw pancreas was fed daily to each of these animals for a week before the absorption test was made.

The data summarized in figure 2 are somewhat surprising in that they show that the absorption of fatty acids is no better than for neutral fat after removal of the pancreas. The solid line curve indicates that in three normal dogs a definite hyperlipemia within three hours was produced by the instillation of 50 cc. of oleic acid into the duodenum. No such hyperlipemia was secured in the depancreatized animals even with the feeding of raw pancreas.

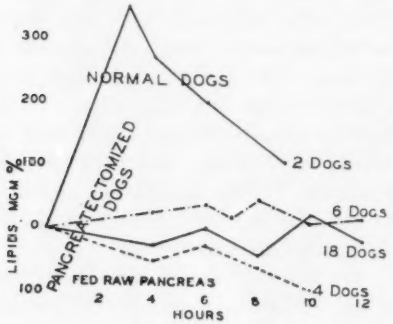

Fig. 1

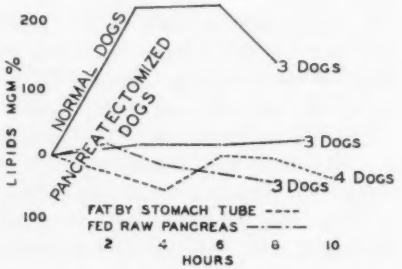

Fig. 2

Fig. 1. Average deviation of blood lipids from 0 hour level after administration of 50 cc. olive oil by stomach tube to normal and pancreatectomized dogs.

Fig. 2. Average deviation of blood lipids in dogs from 0 hour level after administration of 50 cc. oleic acid to unanesthetized normal and pancreatectomized dogs. Oleic acid instilled into duodenum per catheter except in one series of 4 pancreatectomized animals in which it was given by stomach tube.

Absorption of neutral fat and fatty acid in depancreatized dogs as determined by analyses of the feces. In this experiment pancreatectomized dogs which had recovered from operation were placed on a "fat-free" diet containing potatoes, carrots, casein, sucrose, skimmed milk, brewer's yeast and defatted bone meal. On this diet each animal obtained only 8 grams of fat per day. After two to three days on this "fat-free" diet the animals were given olive oil well mixed with the basic meal. After a period of two to four days the oil addition was discontinued and only the basic diet given. Then again for a period of two to four days oleic acid was added to the diet. In some animals the procedure was reversed, the oleic acid being given first and olive oil in the second period. The addition of carmine to the food at the beginning of each change of feeding made it possible to collect the feces corresponding to the periods of olive oil or oleic acid feeding with reasonable accuracy. The defatted bone meal was added to the food to produce stools sufficiently formed for easy collection. Any food left uneaten was weighed and subtracted from the total weight given and cor-

rection made for the amount of oil actually eaten. In some instances this calculated uneaten fat was checked by actual extraction of fat in the Soxhlet apparatus. The stools of each dog for an entire period were combined, a fine suspension in alcohol was prepared in a rotary mixer, and an aliquot of the suspension was dried. The fat was extracted with alcohol and alcohol-ether in a Soxhlet apparatus. The fat was re-extracted with petroleum ether and weighed after drying. Checks on the fat content of the food were similarly made.

TABLE 1

DOG	TIME AFTER OPERATION	FEEDING PERIOD	TOTAL FOOD FAT	OLIVE OIL TAKEN	OLEIC ACID TAKEN	STOOL FAT	PER CENT ABSORPTION OILVE OIL	PER CENT ABSORPTION OLEIC ACID
Comparative absorption olive oil and oleic acid in pancreatectomized dogs								
		days	*grams*	*grams*	*grams*	*grams*	*per cent*	*per cent*
1	1 yr.	4	29.3	342		143.3	61.5	
		4	27.8		241	110.5		58.9
2	2 mo.	4	28.6	305.2		80	76.0	
		4	26.6		182	53.5		74.4
3	1 yr.	4	29.3	171		133.8	33.0	
		4	29		151	77.5		57.0
4	3 mo.	3	17	110.6		70.8	46.0	
		3	14		91	16.9		83.6
5	4 mo.	3	20	128.6		127.2	14.2	
		3	20		126	95.8		35.4
6	1 mo.	3	15	101.3		71.7	38.3	
		3	20		126	43.9		70.0
7	1 mo.	3	15	98.0		44.0	61.1	
		3	20		126	47.2		67.8
						Average	47.2	63.7
Comparative absorption olive oil and oleic acid in normal control dogs								
8		4	29.3	171		1.9	99.05	
		4	29.3		151	11.0		93.9

The data are presented in table 1. The normal animal absorbed 99 per cent of the olive oil and 94 per cent of the oleic acid fed. This agrees very well with the results of other investigators. In the pancreatectomized dog, however, considerable variation was found. One animal absorbed 76 per cent of the olive oil, another only 14.2 per cent, and the remainder amounts intermediate between these extremes. Similar variations occurred in the absorption of oleic acid, i.e., from 83.6 to 34.4 per cent of the amount fed. In three of the animals there was no appreciable difference between the absorption of olive oil and oleic acid. In the remaining four, however, significantly better absorption of oleic

acid took place, i.e., 33 versus 57 per cent, 46 versus 83 per cent, 14 versus 34 per cent, and 38 versus 70 per cent.

Discussion. According to one theory of fat absorption widely held at the present time (7) glycerides in the food are emulsified by the bile and split into fatty acids and glycerol by lipolytic enzymes derived chiefly from the pancreatic juice. The fatty acids then form a water soluble, freely diffusible, combination with bile salts and enter the intestinal mucosa in this form. The compound with bile salts is then broken up and the fatty acid is set free. Glycerol passes independently into the intestinal mucosa, recombines with the fatty acids, and the neutral fat thus formed passes into the lymph. The impairment in fat absorption following pancreatectomy and the fact that fatty acid is on the whole somewhat better absorbed than neutral fat, are in harmony with this view. Nevertheless, the remarkable absorption of neutral fat in some depancreatized dogs which may amount to from 61 to 76 per cent of the fat in the diet, indicates either that this hydrolysis is not essential or else that sufficient lipase is furnished by the gastric and intestinal juices. Improvement in fat absorption with longer survival after operation is not apparent in our data. The decided impairment in the absorption of fatty acid after pancreatectomy is somewhat surprising and may indicate that the pancreas plays some rôle in the absorption of fat other than through the digestive action of pancreatic lipase.

The disappearance of the normal hyperlipemia in response to the oral administration of neutral fat or fatty acid in depancreatized dogs is doubtless due to the impaired and perhaps delayed absorption indicated by the studies on the fat in the feces. This finding may possibly have some significance in the diagnosis of pancreatic disease.

CONCLUSIONS

1. Temporary hyperlipemia, which may be produced in normal dogs by the oral administration of neutral fat or fatty acid, is abolished by removal of the pancreas and is not restored by the administration of active pancreatic juice or raw pancreas.

2. Pancreatectomy produces a varying degree of impairment in the absorption of neutral fat, but some animals may still absorb 75 per cent or more of the fat in the diet.

3. Pancreatectomy produces a definite impairment in the absorption of fatty acid, though not so great as in the case of neutral fat.

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